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The Journal of Clinical Investigation

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

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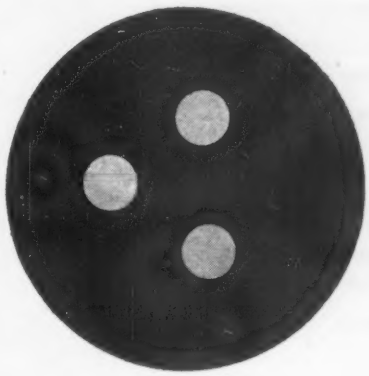

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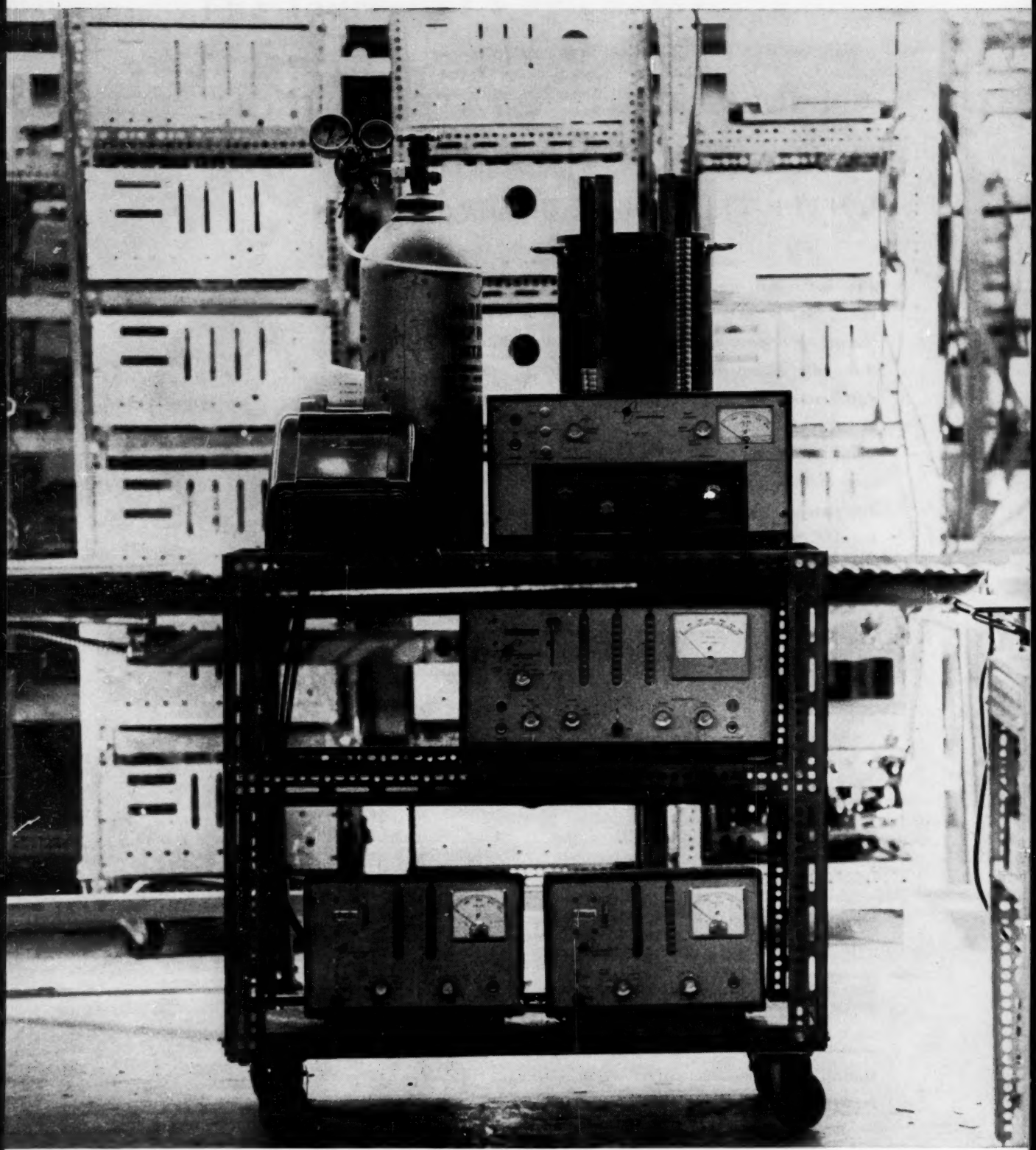

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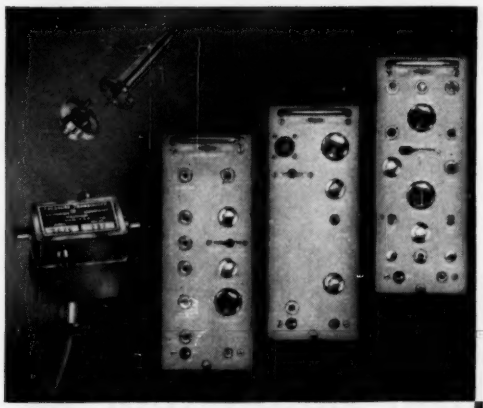

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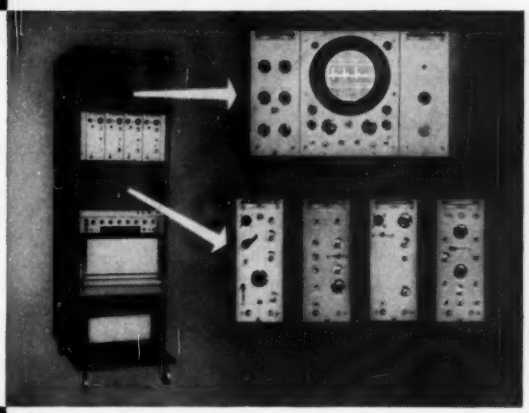

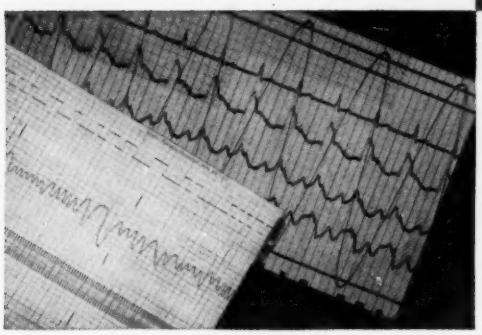

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IT HAPPENED THIS MONTH...

a glance at yesterday in relation to today

IN MAY—(1878)—W. Kühne, of Heidelberg, discusses the recently discovered photosensitive pigments of the retina. "If one considers the extremely widespread occurrence in the animal kingdom of the black pigment of the eye, and other similarly stable pigments, it is scarcely possible to repress the idea that these, in addition to visual purple, also represent visual excitants. . . . Now as I have convinced myself by prolonged observations, partly on myself, and partly on animals . . . that even after the disappearance of the visual purple acute vision is still perfectly possible, I have come to the hypothesis that the visual purple, which is the most unstable visual excitant known at the present time, serves for the perception of feeble light, while the other pigments whose occurrence has been observed in the eye serve for that of more intense light."[1]

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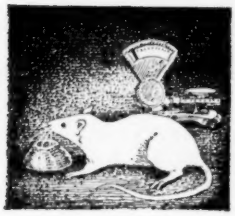

IN MAY—(1943)—Rose and his associates at Urbana, Illinois continue their important experiments on the role of amino acids in human nutrition. Threonine, leucine, isoleucine, and phenylalanine are reported to be necessary constituents of the diet of man. Although histidine was previously shown to be essential for rats and dogs, it is apparently not required for maintenance of nitrogen equilibrium in humans.[2]

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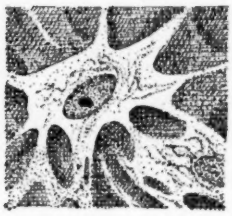

IN MAY—(1954)—Healy, Fisher and Parker[3] of the University of Toronto describe a chemical method for estimating cell multiplication in tissue cultures. The procedure is based upon the determination of desoxyribonucleic acid phosphorus (DNAP). It involves a modified Schmidt and Thannhauser separation of DNA from RNA, followed by the spectrophotometric estimation of the orthophosphate. Good correlation is found between nuclear counts and DNAP values in natural and synthetic media.

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1. Kühne, W.: Addition to the article "On the stable colours of the retina." J. Physiol. *1*:189, 1878-1879. 2. Rose, W. C.; Haines, W. J.; Johnson, J. E., and Warner, D. T.: Further experiments on the role of the amino acids in human nutrition. J. Biol. Chem. *148*:457 (May) 1943. 3. Healy, G. M.; Fisher, D. C., and Parker, R. C.: Nutrition of animal cells in tissue culture. VIII. Desoxyribonucleic acid phosphorus as a measure of cell multiplication in replicate cultures. Can. J. Biochem. Physiol. *32*:319 (May) 1954.

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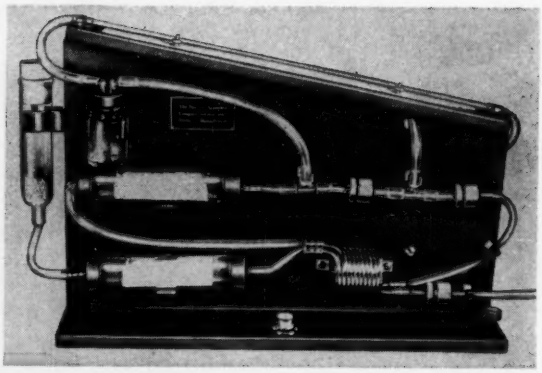

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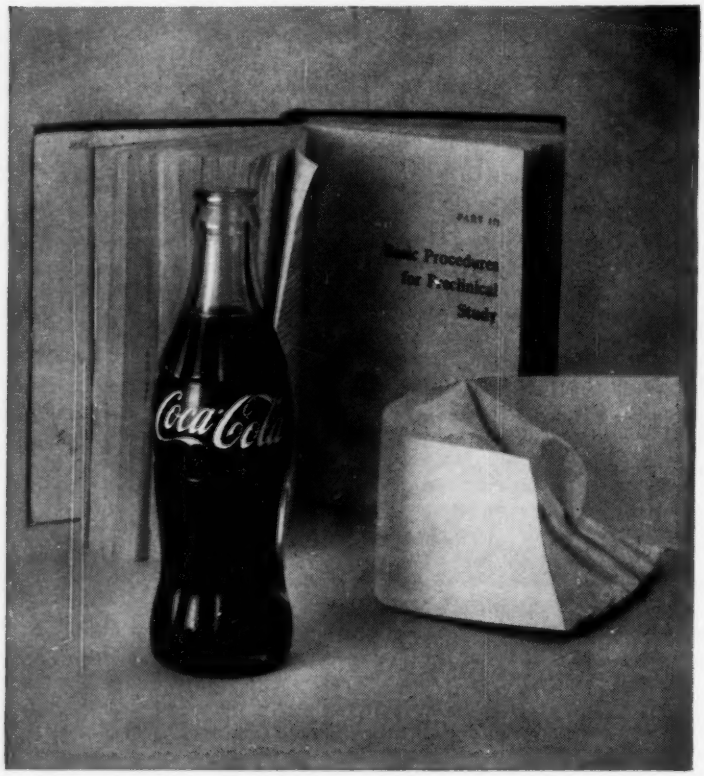

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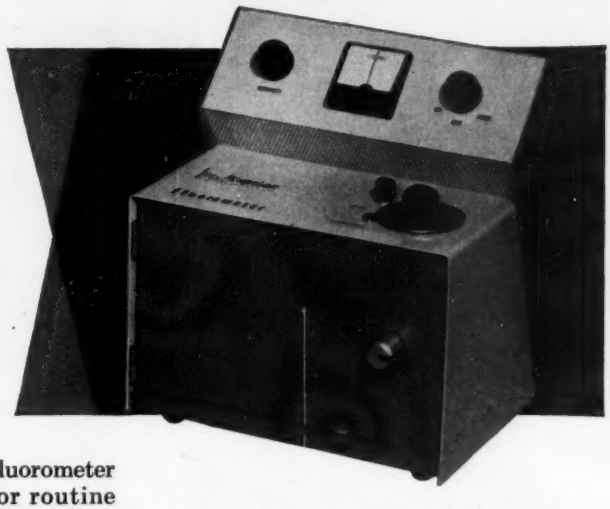

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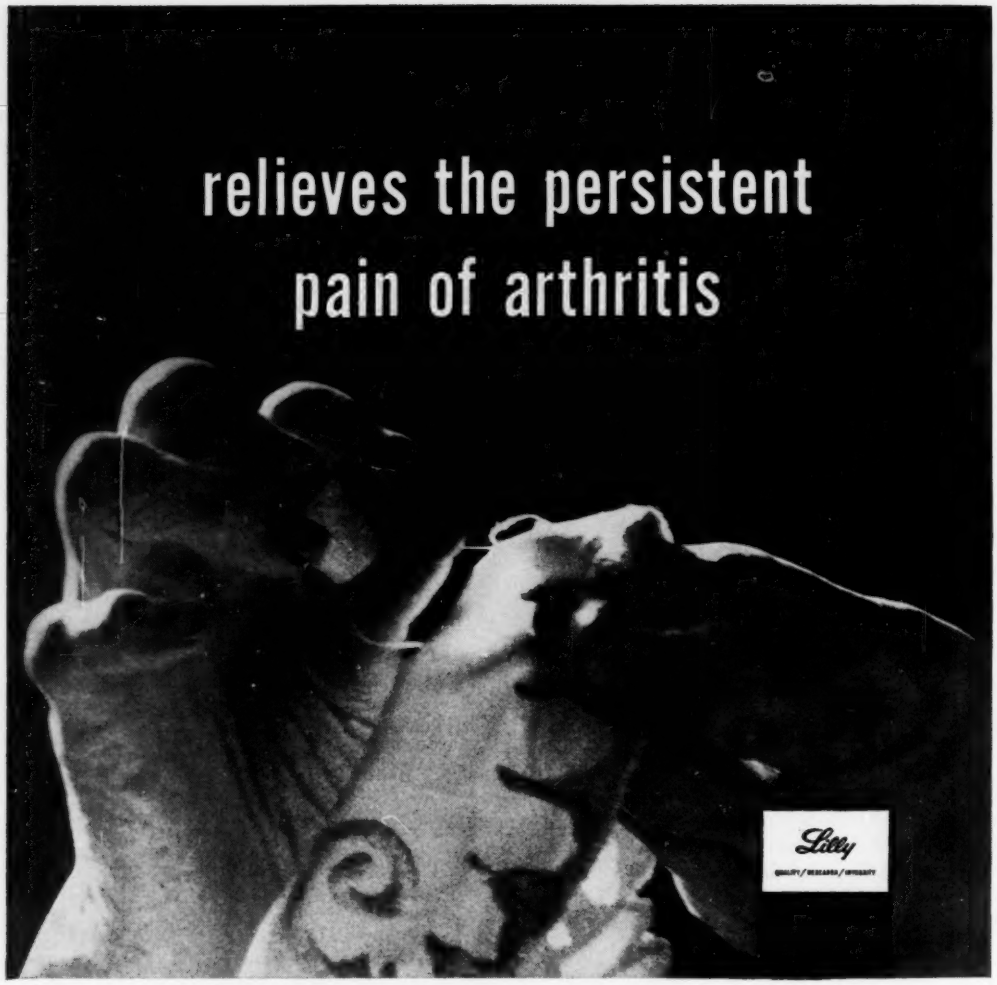

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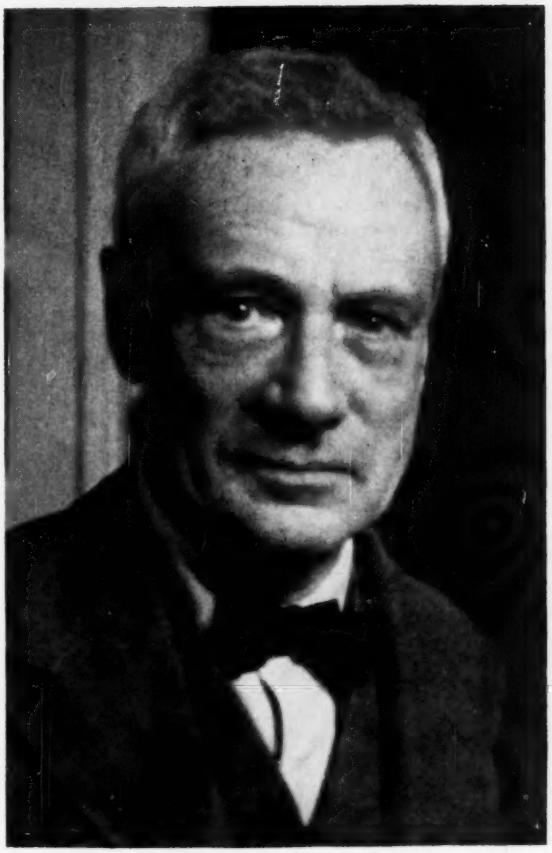
James Lawder Gamble, formerly Editor-in-Chief of *The Journal of Clinical Investigation* and a member of its Editorial Board, died in Boston on May 28, 1959, in his seventy-sixth year. Born in Millersburg, Bourbon County, Kentucky on July 18, 1883, he received his schooling there, his college education at Leland Stanford University (A. B. 1906) and attended Harvard Medical School, graduating M. D. *cum laude* in 1910. Following a two year internship in medicine at the Massachusetts General Hospital and six months in pediatrics at the Children's Hospital, he began his career as a clinical investigator under Dr. Fritz Talbot at the Massachusetts General Hospital. During this period, his future course was very much influenced by Professor L. J. Henderson, as he described in his speech of acceptance of the Kober Medal:



JAMES LAWDER GAMBLE—1883-1959

> Although I never worked directly with Henderson, I learned from him to admire the beauty of the physicochemical systems which sustain the body fluids, the marvel of their automaticity and their remarkable resiliency in the presence of obstacles imposed by disease, and ever since I have been content to work in this wide field as an humble artisan applying the simple tool of quantitative description to the pattern provided by this great architect of concept.

In 1915 he moved to Baltimore and joined the new Department of Pediatrics under Dr. John Howland at Johns Hopkins, a "lush meadow of opportunity," where he formed lasting friendships with Kenneth Blackfan, Edwards Park, McKim Marriott and others who were to become the leaders of American pediatrics. Against the advice of Dr. Howland, to whom he was personally devoted, he decided to give up all formal clinical responsibilities in order to devote his life "to the study of disease by the methods of chemistry." During this period he and Blackfan proved that infants synthesize cholesterol and he obtained fundamental information upon "the Metabolism of Fixed Base during Fasting," by seizing the opportunity provided by an attempt to confirm the observations of a physical culturist that starvation would eliminate epileptic seizures in children.

In 1922, when Oscar Schloss was appointed to the chair of Pediatrics at Harvard, he brought Dr. Gamble to Boston as Assistant Professor and had laboratories constructed for him at the Children's Hospital. Here Dr. Gamble spent the remainder of his life "as a student of disease"; he was promoted to Associate Professor in 1925, to Professor in 1932, and retired as Professor *Emeritus* in 1950, but until the time of his death continued to come to the laboratory to guide the investigations of the "oncoming young men," in whom he took such a keen interest. Dr. Schloss returned to New York after only a brief period in Boston, but Kenneth Blackfan succeeded him and thus began an extremely happy and productive relationship, to use Allan Butler's words, between "Blackfan, the superb clinician and tactful administrator, and Gamble, the gifted investigator," which ended with the former's untimely death in 1941.

Gamble's investigations concerned the mechanisms of acidosis, the disturbances in the body fluids produced by various lesions of the gastrointestinal tract and kidneys and the physiological defences against dehydration, acidosis, and starvation. As early as 1933, with Allan Butler and Charles F. McKhann, he pointed out the importance of intracellular fluid loss and potassium deficits in diarrheal dehydration but his primary concern was with extracellular fluid. Probably his most influential publication, printed in looseleaf form by the Harvard Press, was his syllabus, "Chemical Anatomy, Physiology, and Pathology of Extracellular Fluid." During the war he collaborated with Allan Butler's group at the Massachusetts General Hospital in their clearly conceived studies of a group of conscientious objectors who voluntarily submitted to deprivation of food and water in order to simulate the problems of survivors on a life raft at sea. Based on these studies, Dr. Gamble gave a Harvey Lecture, entitled "Physiological Information from Studies on the Life Raft Ration," which admirably illustrates his extraordinary talent for clear analysis and lucid presentation of experimental data. Following the war, with Wil-

liam Wallace and a group of younger colleagues, he devoted himself to a study of the chemical phenomena of growth and to the causes of some of the inconsistencies of metabolic balance data.

He was not a prolific author; his entire bibliography over a period of 45 years includes only 54 papers, but each one is characterized by clarity of thought, preciseness and lucidity of expression, and a vivid and often whimsical choice of phrase, which were the product of hours of longhand composition and numerous revisions. His data were usually presented in very clear charts, which he claimed to have devised to make up for his mathematical deficiencies but which were a boon to his audiences and a model to be copied by aspirants for election to the Society for Clinical Investigation.

Although his chosen place of work was in a department of pediatrics, his laboratory was sought as a place for training by aspiring young investigators from medicine and surgery as well as pediatrics, many of whom now hold important academic positions in this country and abroad. It was a happy place to work, a far cry from the high-powered research institute, a laboratory in which the neophyte was set to learn by formulating his own problem, by making his own measurements, by informal discussion with his colleagues, and by the final effort to present his data in a simple, precise fashion which could pass Dr. Gamble's kindly but rigorous editorial scrutiny.

Dr. Gamble was the recipient of many honors in recognition of the extent to which his quantitative descriptions and lucid analyses had increased medical understanding of the body fluids in health and disease and had thus laid the foundations for rational, quantitative supportive therapy for patients of all ages. Not only did he receive several honorary degrees—M. S. from Yale in 1930, M. D. from Zurich in 1950 and D. Sc. from Chicago in 1952—but he was given the Borden Award of the American Academy of Pediatrics in 1946, the Kober Medal of the Association of American Physicians in 1951, and in 1955 the Moxon Medal of the Royal College of Physicians and the John Howland Award of the American Pediatric Society. He was a member of the American Society for Clinical Investigation, the Association of American Physicians, the Society for Pediatric Research and the American Pediatric Society, of which he was President in 1945, and was one of very few pediatricians ever to be elected to the National Academy of Sciences.

In 1941, Dr. Gamble succeeded Randolph West as Editor of *The Journal of Clinical Investigation,* and carried the *Journal* through the Second World War. He had been a member of the Editorial Board for the preceding seven years, during the last part of Dr. Austin's and all of Dr. West's term as Editor, and he was eminently qualified by the importance and elegance of his own scientific writing to maintain the high standards of this distinguished journal. In taking on this responsibility, he made his first important innovation, the appointment of an Associate Editor, his former pupil, colleague, and close friend, Allan M. Butler, to whom must go a fair share of the credit for Dr. Gamble's accomplishments as Editor. The two men met weekly with Mrs. Margaret Came, Chief Editorial Assistant, and made their decisions about manuscripts and other phases of editorial policy. Dr. Gamble's term as Editor-in-Chief covered the difficult war period. The seven members of the Editorial Board, composed of distinguished *emeritus* members of the Society, each with particular competence in some field of clinical investigation, who had always served as critical reviewers for the *Journal* and thus established its high quality, were all very busy men with heavy commitments to teaching or government service. Partly because of this, and partly because he believed in making the *Journal* one to be edited *by* as well as *for* the Society, Dr. Gamble changed the established editorial policy during his tenure of office. The Editorial Board became less and less active, and instead the members of the American Society for Clinical Investigation were used increasingly as critical reviewers of manuscripts submitted by their peers. In order to advise him upon the individuals to be selected as reviewers for particular manuscripts, and to help with final decisions based upon their reviews, he enlarged the group of Associate Editors to three; Chester Keefer and Eugene Landis joined Allan Butler in this capacity in 1944. Thus, there emerged a new pattern, still being followed by the *Journal,* of an Editor-in-Chief, assisted by a group of two or three Associate Editors, colleagues of his own choosing in his own city, with the reviewing of manuscripts being done by an ever widening circle of individuals who can bring their expert knowledge and critical faculties to bear upon the writings of their peers. It is doubtful whether the manuscripts submitted to any other journal are subjected to such stringent criticism by such a well-informed group. The Editorial Board was not reappointed in 1946 when Dr. Gamble's term as Editor expired, and the pattern of operation which he had established was maintained. Supervision of the *Journal* policies by the Society continues as in the past through the Editorial Committee, consisting of a group of active members who meet with the Editor annually to review the *Journal's* policies and activities.

A second important innovation during Dr. Gamble's Editorship was the publication of groups of papers upon a single broad topic. The first such group occupied a whole issue (Number 4, Volume 23, July, 1944) and comprised papers under the general heading, "Chemical, Clinical, and Immunological Studies upon the Products of Plasma Fractionation." These papers reported various phases of research upon the plasma fractions being developed by Professor E. J. Cohn and his collaborators under a contract, sponsored by the Committee on Medical Research, with the Office of Scientific Research and Development. Despite the inevitable curtailment of some phases of clinical investigation by the war effort, an adequate flow of good manuscripts continued, and Dr. Gamble was particularly anxious to have the *Journal* used as a medium for publication of the results of the government-sponsored research programs in relation to the problems of military medicine. Actually many of these studies were ultimately published in *The Journal*

of Clinical Investigation. Subsequently, several other smaller groups of papers on other phases of plasma fractionation appeared and an important series of eleven papers on the "Clinical Uses of 2,3-Dimercaptanol (BAL)" occupied half of the July, 1946 issue (Volume 25, Number 4). Dr. Gamble felt very strongly that these papers, so intimately related to the war effort, deserved preferential treatment and tried very hard to get the War Production Board to increase its allotment of paper to the *Journal* to permit their publication as a special supplement. He was very incensed when, his request having been turned down, he received a large luxuriously printed advertising brochure from one of the pharmaceutical firms. His goal was only achieved under Dr. Ferris' Editorship after the war was over, when a Symposium on Malaria appeared as a supplement to the May, 1948 issue of the *Journal.*

Despite the war, the affairs of the *Journal* prospered during the period from 1941 to 1946, when Dr. Gamble was Editor. He was able to increase the amount of advertising appreciably, thus strengthening its financial support. The number of subscriptions rose slowly and steadily from 1,046 in 1942 to 1,530 in 1945 (they now number approximately 5,000), as the importance of the *Journal* as a medium for publication of much of this country's best clinical investigation came to be appreciated by a growing body of scientifically trained clinicians. He was particularly concerned that the *Journal* should reach younger men in training, and thus a lower subscription rate for medical students, residents and fellows was inaugurated. In order to increase the number of pages, he succeeded in obtaining a grant of 2,000 dollars a year for three years from the Commonwealth Fund, which permitted the addition of approximately 200 pages of text per year to those which the Society felt it could afford to publish.

Another problem, which has plagued the Society itself, concerned him, namely, the feeling on the part of a relatively small number of members from the western part of the country that their section was being discriminated against, not only in election to the Society but in the acceptance of papers for the *Journal.* He tried to lean over backwards in this matter and to see that the Editorial Committee became representative of all sections of the country as well as of all the main fields of investigation. He was very sensitive to the criticism that the *Journal* should become more clinical and less investigative. Nevertheless, he and his Associate Editors hewed firmly to the line of quality—quality in the formulation and planning of clinical or experimental studies and quality in the precision and clarity with which the results were presented and conclusions drawn. Consequently, the *Journal* maintained its high standards of excellence throughout his term of service as Editor-in-Chief.

When he and his Associate Editors retired in 1946 and the leadership of the *Journal* was handed on to Eugene Ferris in Cincinnati as Editor, with Arthur Mirsky and William Bean as Associate Editors, they inherited a publication which had survived a difficult war period with a fifty per cent increase in subscription, a continuing reputation for excellence in content and form of presentation of material as a result of vigorous critical scrutiny of manuscripts, and new editorial policies, which are still being followed today.

Dr. Gamble's reputation as a medical scientist and as an Editor entitles him to a lasting place of honor among the great contributors to the development of the type of clinical investigation which has been such a prominent feature of academic medicine in this country. Entering medicine at the time when quantitative clinical methods were being introduced into the clinic and when full-time departments were being established, his life spanned what may well appear in retrospect to be the golden age of clinical investigation, when the individual investigator, as opposed to the increasingly prevalent team of today, could learn and apply relatively simple methods to the solution of fundamental problems, if he had Dr. Gamble's genius for clear formulation, critical analysis, and lucid presentation.

Apart from his specific contributions, it was the combination of rare intellect with an unusually appealing character and personality which endeared him to a close circle of devoted friends and to his students. His "influence stemmed from a clarity of mind, a warmth of friendly interest, a simplicity of character and a gentle integrity which never frightened but always uplifted those whom he touched. As his great friend, A. N. Richards, put it, 'There was not a spurious fibre in him.'"

CHARLES A. JANEWAY, M.D.

A METHOD FOR THE CONTINUOUS MEASUREMENT OF CEREBRAL BLOOD FLOW IN MAN BY MEANS OF RADIOACTIVE KRYPTON (Kr^{79})*

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The measurement of cerebral blood flow by means of the nitrous oxide method of Kety and Schmidt (1) or the krypton-85 (Kr^{85}) technique of Lassen and Munck (2) requires a steady state of at least ten minutes, for it is not until the end of such a period that the uptake of the gas by the brain can be estimated indirectly from its concentration in cerebral venous blood. These methods are, therefore, not applicable to the study of rapid or transient changes in cerebral blood flow. If, however, a γ-emitting inert gas such as krypton-79 (Kr^{79}) is employed, its uptake by the brain can be followed by direct measurement of the γ-radiation through the intact skull, thus permitting the continuous measurement of cerebral blood flow during transient or rapid changes before equilibration between brain tissue and cerebral venous blood is achieved.

The description of the development, evaluation and application of such a method is the subject of this report.

METHOD

Theory

Like the nitrous oxide method, the Kr^{79} method is based upon the Fick principle, which can be expressed as follows:

$$\frac{dQ_B}{dt} = CBF(C_A - C_V), \qquad [1)]$$

where dQ_B/dt is the time rate of change of the quantity of Kr^{79} in the brain, CBF is the rate of blood flow through the brain assuming equal arterial inflow and venous outflow, and C_A and C_V are the arterial and representative cerebral venous Kr^{79} concentrations, respectively.

Since accurate measurement of instantaneous rates of change like dQ_B/dt is difficult, it is preferable to integrate Equation 1 between definite intervals, short enough to keep the resolution time of the method small and to permit the approximation that cerebral blood flow had remained constant during the interval but long enough to allow accurate determinations of the change in quantity of Kr^{79} in the brain. Thus, in the integrated and transposed form,

$$CBF = \frac{\Delta Q_B \Big|_{t_1}^{t_2}}{\int_{t_1}^{t_2} (C_A - C_V)dt}. \qquad [2)]$$

Equation 2 states that the cerebral blood flow rate during a given interval of time is equal to the change in brain Kr^{79} content divided by the integrated arteriovenous Kr^{79} concentration difference during the same interval of time. By measuring brain content and arterial and venous concentrations either continuously or repeatedly at fixed successive intervals of time, as, for example, the one minute intervals in these studies, cerebral blood flow can be calculated continuously over successive one minute periods until the rate of change of Q_B and/or the arteriovenous difference become too small to be determined reliably. Equation 2 applies during saturation or desaturation of the brain with Kr^{79}. Furthermore, since Q_B represents total brain Kr^{79} content and not concentration in the cerebral tissues, CBF is a measure of the blood flow to the whole brain rather than to a representative fraction of it, as is the case with the nitrous oxide method.

Q_B, C_A, and C_V are determined by the counting of the γ-radiation of the Kr^{79} and are expressed as counts per minute (cpm) in the brain or cpm per milliliter of blood, respectively. Since the counting rates in brain and blood are determined under different geometric conditions and, indeed, by different counters, counting efficiencies are different for these two types of measurements, and an equivalence factor (F) obtained by counting a standard Kr^{79} source under the two conditions in a manner to be described below must be included in the numerator of Equation 2.

* Opinions or conclusions contained in this report are those of the authors and are not to be construed as necessarily reflecting the views or endorsements of the Navy Department.

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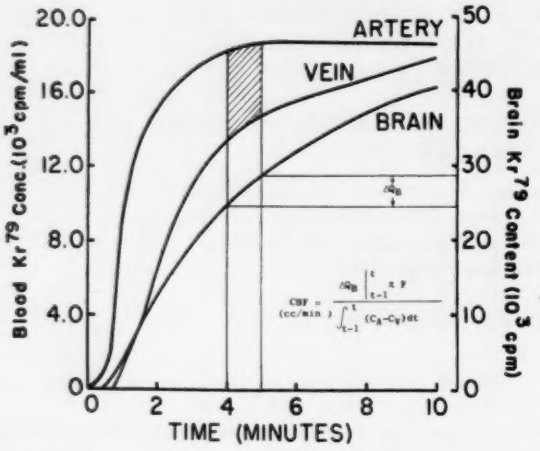

FIG. 1. TYPICAL CURVES FOR ARTERIAL AND INTERNAL JUGULAR VENOUS Kr^{79} CONCENTRATIONS AND BRAIN Kr^{79} CONTENT DURING SATURATION WITH THE RADIOACTIVE GAS. The formula and the method for the calculation of CBF during a one minute interval (fifth minute) are illustrated. ΔQ_B during the fifth minute is the numerator of the formula and is obtained as indicated; the shaded area between the arterial and cerebral venous curves represents the integrated arteriovenous difference during the same interval of time, and is the denominator of the formula. F is a proportionality constant which converts blood and brain counting rates to equivalent units (see text). To obtain continuous minute-to-minute values for cerebral blood flow, the calculation is repeated for each successive one minute interval of time.

The application of this equation to the calculation of cerebral blood flow is illustrated in Figure 1.

Properties, preparation and administration of Kr^{79}. Kr^{79} was chosen as the tracer substance for this method because it is a chemically inert gas which diffuses freely across the blood-brain barrier, emits γ-radiation which permits its direct measurement in brain by means of external counting techniques, and has a suitably short physiological half-life. Its physical half-life is 34 hours (3), and its decay scheme includes K-capture, positron emission, and at least 15 γ-transitions ranging in energy from 44.5 to 833.4 Kev (4). It was produced for these studies by deuteron bombardment of potassium bromide in a cyclotron [1]; the Kr^{79} formed by the ensuing Br-d-2n reaction is trapped in the crystal structure of the bromide. Other radioactive isotopes (Br^{80} and Br^{82}) are also formed in the process. The occluded Kr^{79} is released by solution of the potassium bromide in bromine water in an enclosed atmosphere containing air and a barely measurable volume of inert carrier krypton. The bromine water is then completely distilled into a dry ice-acetone cold trap; the overlying gas is passed through a sodium hyposulfite scrubber to remove any residual radioactive bromine and finally collected in an evacuated 1 L tonometer. The yield of Kr^{79} was assayed by measurement of the γ-radiation from the tonometer at a distance of 100 cm with an ionization chamber type survey meter which had previously been calibrated with a known Kr^{79} source.[2] The quantity of Kr^{79} obtained in the tonometer generally approximated 5 mc.

The Kr^{79} was administered to the subjects in the inspired air which was prepared by flushing the contents of the tonometer into a spirometer and diluting with room air to a final concentration of 100 μc per L. Administration was usually through a non-rebreathing system with collecton of the expired air in a Douglas bag, but in a few cases to be discussed below, rebreathing with CO_2 absorption and O_2 replacement was employed. Under the conditions of these experiments, the highest radiation dosage is in the lungs and has been calculated to be less than 0.1 rad (5).

Measurement of Kr^{79} *content in brain.* The quantity of Kr^{79} in the brain is measured by means of a scintillation counter precisely located with respect to the brain and carefully shielded to exclude radiation from all bodily areas except the brain and overlying extracerebral tissues. The scintillation probe consists of a cylindrical, thallium-activated sodium iodide crystal, 4 cm in diameter and 10 cm in length, a Dumont 6292 photomultiplier tube, and a cathode-follower type pre-amplifier, all enclosed within a light-proof aluminum shield. With the subject in the supine position, the probe is placed laterally with respect to the head so that its proximal end is approximately 25 cm from the midsagittal plane of the head and its longitudinal axis is directed at the midpoint of the line connecting the opening of the external auditory meatus and the top of the crown (Figure 2). The entire probe is surrounded by a 2 inch thick lead cylinder, 12 inches in diameter (Figure 2). Between the side of the head and the proximal end of the lead cylinder, but flush against both, is a 2 inch thick flat lead plate which is cut in the shape of the contours of the brain as "seen" by the scintillation probe (Figure 2). The lead shield was designed to exclude from the probe all Kr^{79} radiation from the room, body and head except that of the brain and directly overlying extracerebral tissues; its design was determined by experiments with a plastic model of the head containing multiple compartments representing brain, extracerebral tissues, and respiratory passages which could be filled with Kr^{79} solutions or gas mixtures as indicated.

The output pulses of the scintillation probe are first amplified (Atomic Instrument Co. Linear Amplifier Model 204-B) and then counted in a decade scaler (Atomic Instrument Co. Model 1070A) equipped with a pulse height discriminator adjusted to the minimum level necessary to exclude all tube noise and other electronic artifacts. Continuous recording of the counting

[1] Kr^{79} was produced for these studies in the Cyclotron Laboratory of the Massachusetts Institute of Technology.

[2] This Kr^{79} source was standardized for us by Dr. Sherman Frankel, Department of Physics, University of Pennsylvania.

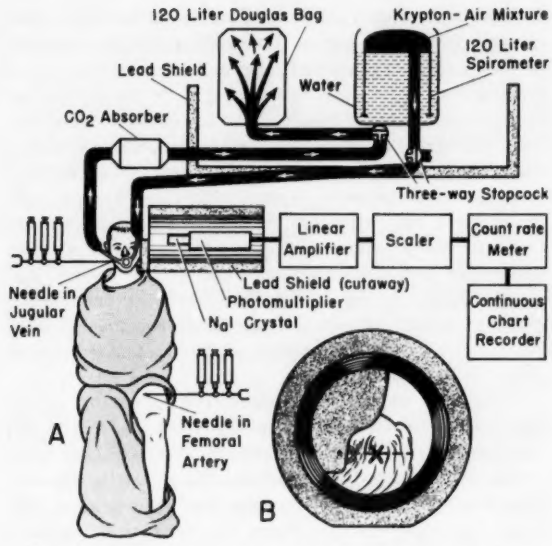

FIG. 2. DIAGRAMMATIC REPRESENTATION OF EXPERIMENTAL SETUP. A, overall view of physical arrangement of subject, lead shields and apparatus; B, view of head as seen by scintillation counter in lead shield with the X marking the point at which the longitudinal axis of the scintillation crystal is directed.

rate is achieved by photography of the scaler face and timer or by differentiation of the scaler output by means of a count rate meter, the output of which is recorded on an Esterline-Angus recording milliammeter.

Measurement of Kr^{79} concentration in blood. If three complete counting channels are available, it is possible to monitor the arterial and cerebral venous Kr^{79} concentrations continuously and simultaneously with the counting from the brain. This can be accomplished by the continuous sampling of blood at a constant rate through glass helices fixed in the wells of well-type scintillation counters. In the present studies, however, discrete, timed arterial and cerebral venous blood samples were drawn into oiled, heparinized syringes at intervals as close to one minute as possible, and their Kr^{79} concentrations were assayed subsequently by measurement of the counting rates in aliquots of each of them transferred consecutively to a single glass helix-scintillation counter system. Each blood sample was mixed by shaking with mercury and introduced into the helix via one of the two-way stopcocks attached by plastic tubing to each opening of the helix. These stopcocks were closed when the system was filled, and by transferring an excess of blood through the helix, anaerobic conditions were maintained for the sample being assayed. The use of the helix also assured a constancy of sample volume and geometric conditions. Between samples the helix was flushed with water, then isopropyl alcohol, and finally dried by a current of air. Sufficient sample counts were collected to yield a coefficient of variation of less than 3 per cent, and corrections were made for background and also for radioactive decay from the time of the head counting procedure.

Determination of the factor F. The factor, F, described previously in the discussion of the theory, is actually a proportionality constant between the overall brain and blood counting efficiencies and can be defined as follows:

$$F = \frac{B}{S} \qquad [3)]$$

where B equals the counting rate from a given quantity of Kr^{79} in blood counted under the blood counting conditions and S equals the counting rate from the same quantity of Kr^{79} distributed throughout the brain and counted under the brain counting conditions.

Since S cannot readily be determined *in situ*, a Plexiglas phantom model of the head was constructed which contained separate "brain," "airway," and "extracerebral" compartments and conformed to average anthropological measurements for adult Caucasian males. The phantom model was utilized in the following manner. A 10 ml syringe containing 5 ml of an aqueous Kr^{79} solution was counted at various distances between 20 and 95 cm from the end of the scintillation crystal employed in the measurement of Kr^{79} in the brain. The contents of the syringe were than quantitatively transferred into the water-filled "brain" compartment of the Plexiglas model; the "extracerebral" compartment was filled with Kr^{79}-free water. Counting rates from the brain model were then measured at various distances from the scintillation crystal which was directed at the same point in the model as in the measurements from the head described previously (Figure 2). The ratio of "brain" to syringe counting rates was thus determined at various distances between the center of each and the end of the probe. It was found not to change systematically between 25 and 95 cm and to average 0.67 over that range (Figure 3). Linear absorption coefficients for the overall γ-radiation of Kr^{79} in water, Plexiglas, brain, and calvarium were also experimentally determined; their values indicated that for the distances involved differences in self-absorption in the model and human head were negligible. It was concluded, therefore, that under the counting conditions described, a given quantity of Kr^{79} distributed in the brain yields a counting rate equal to 67 per cent of its rate at the same distance when distributed in 5 ml of solution contained in a 10 ml syringe. This information makes possible the determination of S in Equation 3 and, therefore, also the factor, F.

Since the efficiencies of the scintillation counters may vary from day to day, F must be determined for each experiment. The procedure is as follows. A concentrated solution of Kr^{79} in blood is made by equilibrating a sample of the subject's blood with a small sample of the undiluted Kr^{79}-air mixture. Five ml of this solution is drawn into a 10 ml syringe; gas bubbles are carefully expelled, and the syringe is capped. Its contents are referred to in subsequent discussion as the "standard" Kr^{79} solution. The syringe is placed at pre-

cisely the same distance from the end of the scintillation crystal as was the midsagittal plane of the head during the cerebral blood flow measurement, and its counting rate is determined under the same conditions and with the identical equipment employed in the measurement of brain Kr^{79} content. A sample of the standard Kr^{79} solution is then transferred to the glass helix and counted with the same equipment and in the same manner as the experimental blood samples.

If N_h and N_s are the counting rates obtained from the standard solution in the helix and in the syringe, respectively, and V_h and V_s are the corresponding volumes of the solution in the helix and the syringe, then from Equation 3 and the results of the experiments with the phantom "brain" model,

$$F = \frac{B}{S} = \frac{N_h/V_h}{0.67\ N_s/V_s} = \frac{N_h\ V_s}{0.67\ N_s\ V_h}. \qquad [4)]$$

Since the arterial and cerebral venous concentrations are determined directly in counts per minute per helix volume, the helix volume, V_h, cancels out when F is introduced into Equation 2.

The final form of the equation from which CBF is calculated is then as follows:

$$CBF = \frac{\Delta Q_B \Big|_{t-1}^{t} \text{ X } N_h\ V_s}{\int_{t-1}^{t} (C_A' - C_V') \text{ X } 0.67\ N_s} \qquad [5)]$$

where C_A' and C_V' are the arterial and cerebral venous concentrations in counts per minute per helix volume.

Procedure

All studies reported here were performed in normal young men in the supine position. Arterial and cerebral venous blood was sampled as previously described (1). After establishment of the sampling systems, the head was carefully placed with respect to the scintillation probe and lead shield as described above.

In all studies Kr^{79} concentration in the inspired air was adjusted to approximately 100 μc per L by dilution in a 120 L spirometer with room air or a mixture of 15 per cent N_2O, 64 per cent N_2, and 21 per cent O_2. The nitrous oxide mixture was employed in several studies in which cerebral blood flow was measured simultaneously by the nitrous oxide technique (1) for the purpose of comparison with the Kr^{79} method. Administration was usually through a non-rebreathing respiratory system. A rebreathing system was used only when the Kr^{79} was insufficient to dilute to the specified concentration and volume necessary to sustain respiration for at least 10 minutes. Cerebral blood flow was then measured only during the desaturation period following 10 minutes of rebreathing of the inspired gas mixture. During the rebreathing, CO_2 was removed by a baro-lime absorber in the expiratory circuit, and oxygen was fed into the spirometer to maintain constant the O_2 tension monitored by a Pauling oxygen analyzer in the inspiratory pathway. A three-way stopcock in the inspired air line permitted rapid switching between room air and spirometer gas as required; a similar stopcock in the expiratory pathway permitted switching to a Douglas bag during non-rebreathing or to the spirometer during rebreathing (Figure 2). To facilitate shielding from the scintillation probe, a rubber mouthpiece rather than a mask was employed for administration of the gas, and the nasal passages were blocked by a nose clip. Dead space was minimized by valves in the inspiratory and expiratory pathways close to the mouthpiece. The spirometer and Douglas bag were placed behind lead X-ray shields as far as possible from the scintillation probe to reduce the contribution to the background from the relatively large quantities of Kr^{79} contained within them (Figure 2).

During all periods of measurement, breathing of the appropriate inspired air, counting from the head, and sampling of arterial and cerebral venous blood were begun simultaneously at the zero time for that period. The first pair of blood samples was drawn at a constant rate of 10 ml per minute for the entire first minute to provide integrated values over that period. Thereafter, timed simultaneous arterial and venous blood samples of approximately 5 ml each were drawn as rapidly as possible (approximately 10 seconds) at intervals of 1 to 1.5 minutes. Measurements during saturation lasted 10 minutes from the onset of breathing the Kr^{79} gas mixture; measurements during desaturation lasted 10 minutes from the onset of inhalation of room air following a 10 minute period of breathing the Kr^{79} gas mixture.

In order to test the ability of the method to detect changes in cerebral blood flow, several studies were performed during the infusion of *l*-norepinephrine, the breathing of 7 per cent CO_2, or hyperventilation. These conditions were introduced only during the desaturation period after several minutes of resting control values had been obtained.

Blood oxygen and carbon dioxide contents were analyzed manometrically by the method of Van Slyke and Neill (6). Blood pH was determined anaerobically at ambient temperature by means of a glass electrode and Beckman Model G potentiometer and corrected to 37° C by the factors of Rosenthal (7). Blood pCO_2 was calculated according to the nomogram of Peters and Van Slyke (8). Mean arterial blood pressure was measured by means of an air-damped mercury manometer connected to the femoral arterial manifold and adjusted to carotid artery level.

RESULTS

By means of the Kr^{79} method, it was possible to follow total cerebral blood flow minute by minute. In Table I are presented the results of 14 studies in 10 normal young men in which measurements were made continuously during steady state periods of approximately 10 minutes. Six of the studies were performed during saturation

and eight during desaturation. During the first minute or two after the onset of saturation or desaturation, the quantity of Kr^{79} in the lungs is changing rapidly. Since it is enormously greater than the amount in the brain, even the small fraction of its scattered radiation which bypasses the lead shield significantly increases the rate of change of the head counting rate and leads to artifactually high values for cerebral blood flow. By the third minute, however, the lung Kr^{79} content is usually changing slowly and only negligibly influences the slope of the head counting curve. After the eighth minute, the head counting curve frequently becomes so flat and the arteriovenous difference so small that reading errors may greatly influence the calculated values for blood flow. Therefore, only the values between the third and eighth minutes were considered reliable and these are included in Table I. Over this period the minute-by-minute values for cerebral blood flow varied relatively little; the overall standard deviation within studies was equal to ± 72 ml per minute or about ± 6 per cent of the mean, and the variance within studies was significantly less than the variance between them ($p < 0.001$). The total mean of all 14 studies was 1,247 ml per minute (SD = ± 277). Mean cerebral blood flow in the ten subjects was 1,236 ml per minute (SD = ± 246). The results of saturation and desaturation studies were not significantly different. A slight but systematic upward drift of the values with time was observed in both the saturation and desaturation studies, but it was considerably more prominent in the former and may, perhaps, have resulted from CO_2 accumulation in the dead space of the respiratory system.

The Kr^{79} and nitrous oxide methods were compared simultaneously in nine experiments in eight subjects; the inspired air mixture then contained 15 per cent N_2O in addition to the Kr^{79},

TABLE I
Minute-to-minute cerebral blood flow in normal young adult men

Subj.	Type of study*	Cerebral blood flow (*ml/min*) During following time in minutes: 3	4	5	6	7	8	Mean ± SD
GM	*S*	946	1,042	1,074	957	1,073		1,018 ± 59
NP	*S*	1,156	1,156	1,214	1,296	1,327	1,311	1,243 ± 74
JB	*S*	1,599	1,632	1,555	1,519	1,509	1,443	1,542 ± 71
DT	*S*	1,188	1,182	1,337	1,250	1,352		1,262 ± 70
HC	*S*	1,012	1,038	1,197	1,294	1,276	1,209	1,171 ± 109
HC	*D*	864	771	841	817	828	863	827 ± 69
JL	*S*	936	983	986	938	1,027	1,083	992 ± 53
JL	*D*	1,033	932	897	858	866	879	911 ± 58
GD	*D*	1,000	1,133	1,116	1,084	1,090	1,112	1,089 ± 45
SK	*D*	995	1,027	1,090	1,143	1,169	1,291	1,114 ± 99
JS	*D*	1,553	1,643	1,631	1,627	1,726	1,633	1,635 ± 58
JS	*D*	1,438	1,362	1,395	1,430	1,436	1,423	1,413 ± 46
EP	*D*	1,605	1,695	1,675	1,592	1,536	1,527	1,605 ± 72
EP	*D*	1,505	1,539	1,640	1,730	1,680	1,700	1,632 ± 81
				Total 14 studies				
Mean		1,202	1,224	1,261	1,252	1,278	1,289	1,247
SD								±277
				10 Subjects				
Mean		1,186	1,216	1,252	1,239	1,271	1,290	1,236
SD								±246
				6 Saturation studies				
Mean		1,140	1,172	1,227	1,209	1,261	1,262	1,205
SD								±200
				8 Desaturation studies				
Mean		1,249	1,263	1,286	1,285	1,291	1,304	1,278
SD								±334

* *S* = saturation, *D* = desaturation.

TABLE II

Comparative cerebral blood flow values calculated by indirect method from integrated cerebral arteriovenous N_2O and Kr^{79} differences

Subj.	Cerebral blood flow (*ml/100 g/min*) N_2O	Kr^{79}
GD	55	66
HC	58	59
SK	56	60
JL	54	49
JL	51	50
GM	66	58
NP	48	48
JB	68	60
DT	62	65
Mean	57.5	57.3

Mean overall diff. ± SD = 4.6 ± 3.8

TABLE III

Comparison between the results of the N_2O and direct Kr^{79} methods for the determination of cerebral blood flow

Subj.	Cerebral blood flow N_2O method	Direct Kr^{79} method
	ml/100 g/min	*ml/100 ml/min*
HC	58	86
HC	55*	60
SK	56	80
JL	54	69
JL	51	86
JL	51	63
GM	59*	71
NP	48	82
JB	68	99
DT	62	81
JS	64*	104
JS	58*	89
EP	58*	96
EP	57*	98
Mean	57.1	83.1†
SD	±5.3	±13.7

Coefficient of correlation = 0.55 ($p < 0.05$)

* Calculated from integrated cerebral arteriovenous Kr^{79} differences by indirect method.

† Significantly different statistically as determined by method of paired comparison ($p < 0.001$).

and measurements were made during a steady state of at least ten minutes as required by the nitrous oxide technique (1). Cerebral blood flow was calculated in two ways: *1)* indirectly from the integrated arteriovenous N_2O difference over the entire ten minute period as described by Kety and Schmidt (1); and *2)* indirectly from the integrated arteriovenous Kr^{79} difference exactly as with nitrous oxide. The brain : blood partition coefficient for krypton employed in the latter calculations was 1.06, the average value found by Lassen and Munck (2) in subjects with normal hematocrits. Almost identical values for cerebral blood flow were obtained with both gases when calculated in this manner (Table II); there-

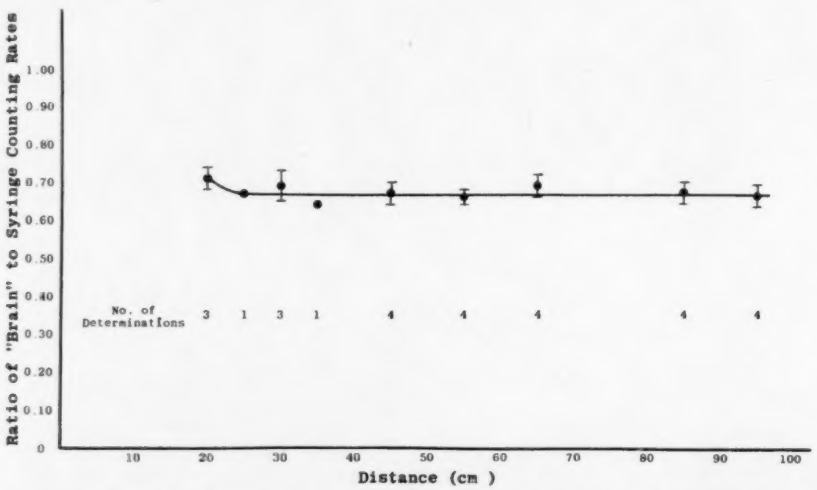

FIG. 3. RATIO OF COUNTING RATE FROM A GIVEN QUANTITY OF Kr^{79} DISTRIBUTED THROUGHOUT SOLUTION IN "BRAIN" COMPARTMENT OF PLASTIC HEAD MODEL TO COUNTING RATE AT SAME DISTANCE FROM SAME QUANTITY OF Kr^{79} CONCENTRATED IN 5 ML OF SOLUTION IN A 10 ML SYRINGE AS A FUNCTION OF DISTANCE BETWEEN THE MIDSAGITTAL PLANE OF THE HEAD OR THE MIDDLE OF THE SYRINGE AND THE PROXIMAL END OF THE SCINTILLATION CRYSTAL (SEE TEXT).

fore, both methods were considered equally valid for comparison with the direct minute-to-minute method which was applied simultaneously in seven of the above experiments as well as in seven additional studies, including six in which Kr^{79} alone was employed. In order to compare the indirect method, which measures blood flow per 100 g of brain, taken as a whole, per minute, with the direct method, which yields total cerebral blood flow per minute, endocranial volume was calculated from extracranial measurements by the method of Lee and Pearson (9). No corrections were made for endocranial volume *not* occupied by the brain or for the specific gravity of the brain. The average of the minute-to-minute blood flow values between the third and eighth minutes per 100 ml endocranial volume was then compared with the blood flow per 100 g brain per minute obtained by the indirect method over the entire ten minute period (Table III). The values obtained with the direct method uniformly exceeded those of the indirect technique (mean values equal 83.2 ml per 100 ml per minute and 56.9 ml per 100 g per minute, respectively), but the correlation between them was statistically significant ($R = +0.55$; $p < 0.05$).

In five studies cerebral blood flow was experimentally altered in the midst of the measurements to test the ability of the Kr^{79} method to detect and follow rapid changes. All measurements were made during desaturation, and the experimental conditions were imposed after a two minute control period. The studies included two on the effects of breathing 7 per cent CO_2 and one each on the effects of hyperventilation of room air, hyperventilation of 100 per cent O_2, and the intravenous infusion of *l*-norepinephrine at a rate of approximately 16 μg per minute. The results are graphically represented in Figure 4 and demonstrate the ability of the Kr^{79} method to follow continuous changes in cerebral blood flow. In Table IV are compared the maximum percentage changes in cerebral blood flow caused by these procedures with those previously observed in nitrous oxide method studies (10, 11). Although the experimental conditions cannot be compared precisely, the direct Kr^{79} method appears to be at least as sensitive as the nitrous oxide technique and to yield proportionate results.

TABLE IV

Comparative changes in cerebral blood flow during various procedures as measured by direct Kr^{79} and N_2O methods

Procedure	Per cent change in cerebral blood flow		
	Kr^{79} method	N_2O method (ref. no.)	
Inhalation, 7% CO_2	+86	+75	(10)
Hyperventilation, room air	−31	−34	(10)
Hyperventilation, 100% O_2	−33		
l-Norepinephrine infusion	−28	− 8	(11)

DISCUSSION

Total cerebral blood flow values obtained with the Kr^{79} method exceed those to be expected from the results of the nitrous oxide technique in a representative fraction of brain and the brain weight. This discrepancy is probably attributable to the collective effect of a number of factors. Although carefully shielded from the remainder of the body, the scintillation counter measuring brain Kr^{79} content must also detect radiation from the extracerebral tissues overlying the brain. Changes in Kr^{79} content of these tissues contribute to the slope of the head count curve and, therefore, also to the calculated values for cerebral blood flow. In order to evaluate the contribution from the overlying soft tissues, a tourniquet was applied to the head just above the ears, and the subject was allowed to breathe Kr^{79}. After six minutes the tourniquet was released; despite a noticeable reactive hyperemia of the scalp, there was no significant change in the slope of the head count curve. These results indicate negligible contributions from the scalp probably because of its smaller volume and lower blood flow than the brain; the contribution from the skull remains unevaluated but is probably similar. The extracerebral tissues may, perhaps, account for some of the discrepancy between the Kr^{79} and nitrous oxide methods but apparently do not obscure the measurement of changes in cerebral blood flow. For example, the directions and degrees of change elicited by hyperventilation and carbon dioxide inhalation are almost identical to those observed with the nitrous oxide technique (Table IV), despite the fact that these procedures cause opposite changes in extracerebral blood flow (12).

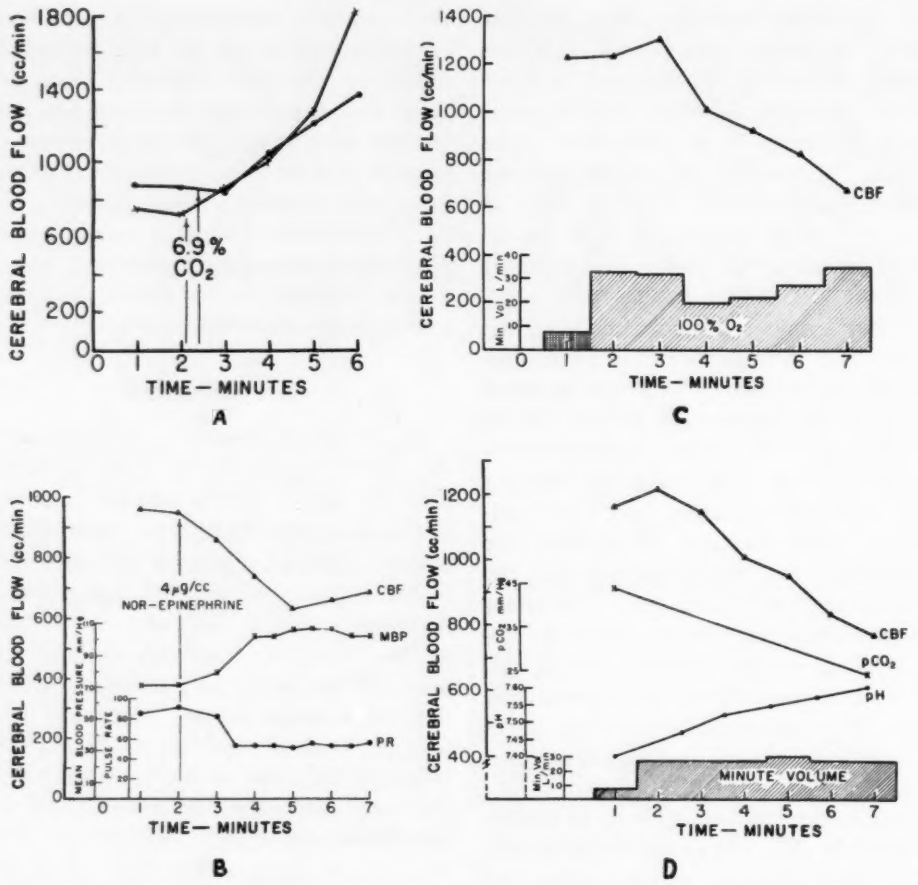

FIG. 4. MINUTE-TO-MINUTE VALUES FOR CBF AS DETERMINED BY THE Kr^{79} METHOD DURING EXPERIMENTALLY INDUCED CHANGES IN CEREBRAL BLOOD FLOW. A, two studies during the inhalation of 7 per cent CO_2; B, during the continuous intravenous infusion of 4 μg per ml *l*-norepinephrine solution at a rate of approximately 4 ml per minute; C, during hyperventilation of 100 per cent O_2 at a rate of approximately 30 L per minute; D, during hyperventilation of room air at a rate of approximately 30 L per minute.

Rapid changes in the Kr^{79} content of the lungs and respiratory passages also influence the slope of the head count curve. This effect arises partly, perhaps, from incompletely shielded paranasal sinuses but mainly from scattered radiation from the chest. Although both sources represent a very small fraction of the radioactivity in the respiratory system, they may still be significant compared to the quantity in the brain because of the low tissue: gas partition coefficient (approximately 0.05) of krypton (13). As previously noted, this effect is sufficient to invalidate measurements made during the first and often second minutes of saturation or desaturation and may also contribute a little to the excessively high values for cerebral blood flow observed in subsequent minutes. In one experiment a continuous record of chest radioactivity was made throughout the procedure, and its effect on the head counts was calculated by means of a "scattering" factor determined in experiments with "phantom lungs." It was found to increase the calculated value for cerebral blood flow during the three to eight minute period by 3.5 per cent.

The greatest single cause of the high values obtained with the Kr^{79} method is probably the disproportionate weighting of different areas of the brain in the total head count. The overall

linear absorption coefficient for Kr^{79} γ-radiation in brain tissue was measured and found to be approximately 0.09 cm^{-1} (one-half thickness = 8 cm); there is, therefore, significant self-absorption of the γ-radiation within the brain. Because of this effect as well as the inverse square law, the Kr^{79} content in the cerebral tissues becomes progressively less represented in the total head counting rate in going from the surface of the brain closest to the counter to the other side. It has been estimated on the basis of theoretical considerations that, even with uniform Kr^{79} concentration throughout the brain, the most proximal 1 cm thickness of brain tissue would alone account for at least 18 per cent of the total counting rate or three times the amount to be expected if all areas were equally represented. Since the most superficial and, therefore, most efficiently represented areas contain disproportionately high amounts of gray matter compared to the brain as a whole, the head counting rate is excessively weighted in favor of the cerebral cortical tissues which have considerably higher rates of blood flow than most other areas of the brain (14). This effect undoubtedly accounts for much of the discrepancy between the results of the Kr^{79} and N_2O methods; it may also render the method susceptible to errors arising from the redistribution of blood flow between the deep and superficial areas of the brain.

Despite its defects, the Kr^{79} method performs quite well in studies of functions not otherwise measurable in man. It permits the estimation of cerebral blood flow under nonsteady state conditions and, as illustrated in Figure 4, is capable of following rapid or transient changes in this function. Although its descriptions of these changes may not be quantitatively accurate, they are indicative of and probably proportional to those actually occurring in the brain. It does not require the assumption of a constant cerebral metabolic rate as does the arteriovenous O_2 difference method which can also be used for the detection of rapid changes in cerebral blood flow (15). In fact, when combined with arteriovenous O_2 differences, the Kr^{79} method can be employed to follow changes in cerebral metabolic rate as well.

Many of the problems encountered in the development of the Kr^{79} method are specifically related to the tracer substance and the form in which it was administered. A nongaseous substance which fulfilled the necessary requirements and which could be injected intravenously would obviate the influence of the isotope in the lungs, air passages and paranasal sinuses with considerable simplification of the problem of shielding. Antipyrine iodinated with I^{131} has been shown (16) to cross the blood-brain barrier rapidly and to have a distribution coefficient between brain and blood of approximately unity (17); it appears to have certain advantages with respect to this method which warrant further investigation.

SUMMARY

1. A method for the rapid continuous estimation of total cerebral blood flow in man by means of radioactive krypton (Kr^{79}) is presented; its theoretical basis, developmental experiments, and procedure are described.

2. Mean total cerebral blood flow in ten normal young men was 1,236 (SD = ± 246); the standard deviation among minute-to-minute values within studies was ± 72 ml per minute or about 6 per cent of the mean value.

3. Cerebral blood flow determined by the Kr^{79} method and corrected for intracranial volume was found to be significantly higher than the values obtained simultaneously with the nitrous oxide method; in 14 experiments the mean values were 83 ml per 100 ml intracranial volume per minute and 57 ml per 100 g brain tissue per minute, respectively. The results of the two methods were, however, significantly correlated ($R = +0.55$; $p < 0.05$).

4. Sources of error in the Kr^{79} method are evaluated, and the causes of the discrepancy between the results of the nitrous oxide and Kr^{79} methods are discussed.

5. The Kr^{79} method was found capable of following rapid changes in cerebral blood flow such as, for example, those induced by hyperventilation, CO_2 inhalation, or *l*-norepinephrine infusion.

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MYOCARDIAL METABOLISM OF FATTY ACIDS *

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The myocardium is able to utilize both carbohydrates and noncarbohydrates for the performance of its work (1). Extraction of an individual substrate is usually determined by its arterial level. Thus, after a carbohydrate meal the heart derives much of its energy from the metabolism of carbohydrates; however, in the fasting state myocardial energy production is dependent upon noncarbohydrates, primarily fatty acids (1).

Recent studies (2, 3) have shown that the principal lipid fraction of plasma concerned with the transport and metabolism of fatty acids is the plasma nonesterified or free fatty acid (FFA) fraction. Previous studies have already demonstrated that the heart uses a considerable amount of FFA (3). The present study was undertaken to determine further the role of fatty acids in myocardial metabolism.

MATERIALS AND METHODS

Most of the methods used in this investigation have been described in detail in previous studies from this laboratory. Coronary sinus blood was obtained from a catheter placed in the coronary sinus, and blood samples from metabolic studies were simultaneously obtained from arterial and coronary sinus blood (1). The coronary blood flow was determined with the nitrous oxide desaturation method (4). Blood glucose was determined by the method of Hagedorn and Jensen (5) using Somogyi's method to prepare the blood filtrate (6). The manometric method of Van Slyke and Neill was used for blood oxygen analyses (7). Total fatty acids (TFA) were determined according to the method of Man and Gildea (8) based on the Stoddard and Drury method (9). According to these investigators, the error of this method is ± 5 per cent. However, modification of their original procedure developed by Siegel (10) resulted in the variance of the difference between two observations of only 0.000232 and a standard error of the difference of 0.015 (Table I). In the procedure employed in this laboratory the entire filtrate resulting from the extraction and deproteinization of 2.0 ml of plasma was used for subsequent hydrolysis. This provided a larger volume used for titration; it also removed possible errors in measurements arising from working with a volatile solution which must be made to a certain volume and from which an aliquot must be taken for subsequent analyses. In addition, all titrations were performed under nitrogen, as originally described by Man and Gildea (8). This resulted in sharper end-points and eliminated the fading effect resulting from exposure to atmospheric carbon dioxide. The data in Table I were obtained prior to the analyses carried out in this modification, in order to insure that the determinations described in this report were truly significant. FFA were determined in duplicate with the recently modified technique of Gordon, Cherkes and Gates (11). Recovery experiments in this laboratory with this technique for stearic, palmitic and oleic acids added to plasma in the concentration of 1 mEq per L indicate 96 to 102 per cent

TABLE I

Plasma fatty acids; agreement of duplicate determinations

Plasma fatty acids		Per cent diff.
Aver.	Diff.	
mEq/100 ml		
0.880	0.010	1.14
0.850	0.010	1.19
0.530	0.000	0.00
0.522	0.002	0.40
1.078	0.008	0.73
0.940	0.020	2.13
0.818	0.020	2.40
0.860	0.010	1.16
1.602	0.002	0.12
1.810	0.010	0.76
1.645	0.005	0.30
1.855	0.025	1.33
1.875	0.005	0.27
0.880	0.010	1.13
0.985	0.005	0.55
0.899	0.010	1.12
1.400	0.020	1.43
0.795	0.005	0.63
0.965	0.005	0.52
0.880	0.010	1.10
	Mean	0.91
	SD	0.61
	SE	0.14

* Supported by United States Public Health Service Grant H-2678, the Life Insurance Medical Research Fund, the American Heart Association, and the Tobacco Industry Research Fund.

TABLE II

Results on fasting patients

Patient	N_2O CBF	Myocardial extractions							Mycardial usage		O_2 extraction ratio†	
		ΔO_2*	Art. TFA	Δ TFA	Art. FFA	Δ FFA	Art. iod. number	Cor. sin. iod. number	TFA	FFA	TFA	FFA
	ml/100 g LV/min	*vol %*	*mEq/ 100 ml plasma*	*mEq/ 100 ml plasma*	*mEq/ 100 ml plasma*	*mEq/ 100 ml plasma*			*mEq/100 g/min*		*%*	*%*
FT	65	10.9	0.989		0.1096							
BB	60	10.7	1.263	0.107	0.1090	0.0060			0.0056	0.0021	50	18
EM	63	14.5	1.052	0.082	0.0913	0.0348			0.0301	0.0128	187	79
WB	84	9.3	1.263	0.056	0.0870	0.0216			0.0261	0.0101	187	72
LL	53	10.1	1.290	0.020	0.0610	0.0190	112	112	0.0059	0.0056	61	59
SL		10.5	2.010	0.083	0.0140	0.0262	114	118				
Mean	65	11.0	1.311	0.0515	0.0780	0.0215	112	115	0.0169	0.0076	145.2	57.2
					Effect of heparin							
JV‡	91	10.8	1.304	0.141	0.0668	0.0198	138	155	0.0836	0.0117	482	67
§			1.175	0.076	0.1370	0.0240	174	195	0.0450	0.0142	260	82
JH‡	46	13.4	1.090	0.019	0.0835	0.0249			0.0052	0.0068	48	63
§			1.033	0.078	0.1207	0.0351	143	159	0.0212	0.0095	195	88
RD‡	(diabetic)		1.101	0.044	0.0780	0.0158	140	144				
§			0.975	0.075	0.1010							
Mean‡			1.165	0.068	0.0761	0.0223	139	149	0.0444	0.0093	264.8	65.15
§			1.061	0.076	0.1195	0.0295	155	172	0.0331	0.0119	227.5	85

* Δ Mycardial extraction, i.e., coronary sinus arteriovenous difference.
† See Footnote 1 in text.
‡ Before heparin.
§ After heparin.

recovery. The blood samples for FFA were drawn into chilled tubes and refrigerated until the determinations were performed, in no case longer than 4 hours after sampling. A modification of the method of Yasuda was used for the iodine number determinations (12). Optical density was measured at 700 mμ with a Coleman junior spectrophotometer.

The usage of fatty acids (milliequivalents per 100 g of heart muscle per minute) was calculated as the product of coronary plasma flow (milliliters per 100 g heart muscle per minute) times the myocardial extraction of fatty acid (milliequivalents per 100 ml plasma). The oxygen extraction ratios of fatty acids were calculated as the ratios of their oxygen equivalent to the myocardial extraction.[1] The oxygen extraction ratio represents the contribution of the aerobic catabolism of fatty acids to the total myocardial oxygen extraction.

The patients listed in Table II had hypertensive, rheumatic or arteriosclerotic heart disease but were not in congestive heart failure. The first group deals with fasting individuals. In the second group, the fasting patients were given orally 2 ounces of a fat emulsion[2] containing 19 per cent saturated and 81 per cent unsaturated fatty acids. However, the metabolic studies reported here were not carried out until 3 to 4 hours after ingestion of this preparation when little or no lipemia remained (Table II). After blood sampling from the initial studies, 10 mg of aqueous heparin was given intravenously and blood was drawn again 20 minutes later for repeat metabolic studies (Table II). The coronary blood flow was determined in the interval between the two sets of blood samples, since studies on dogs, as reported in this paper, indicate that heparin does not alter the coronary blood flow despite the fact that it diminishes lactescence.

Two sets of animal experiments were performed (Tables III and IV). In the first group, myocardial utilization of fatty acids was determined in fasting dogs before and 20 minutes following the intravenous injection of 50 mg of aqueous heparin (Table III). The second group of animals was given 60 ml of Lipomul; 3 hours later the animals were anesthetized and samples were taken before and 1 hour after intravenous injection of 50 mg of heparin (Table IV). In this group, coronary blood flow was measured during lactescence and after clearing of the plasma with heparin (Table IV).

RESULTS

Myocardial fat metabolism in the postabsorptive state. Table II contains the results obtained in a group of six fasting patients. Results of similar studies in a series of 34 fasting dogs are given in Table III. In patients in the postabsorptive state, the range of the arterial levels of total fatty acids was from 0.989 to 2.010 mEq per 100 ml plasma

[1] Oxygen extraction ratio per cent $= \frac{O^2 \text{ equivalent of extracted fat}}{\text{myocardial oxygen extraction}} \times 100.$
Oxygen equivalent of fatty acids = 570 × milliequivalents of myocardial fatty acid extraction.

[2] Lipomul, Upjohn Company.

with a mean value of 1.311 mEq per 100 ml of plasma. The range of myocardial extraction of total fatty acids in these individuals was from 0.017 to 0.083 mEq per 100 ml plasma, while the mean oxygen extraction ratio for TFA ranged from 50 to 187 per cent. In the same group, the arterial levels of FFA varied from 0.014 to 0.1096 mEq per 100 ml of plasma with a mean value of 0.0780 mEq per 100 ml plasma. The mean myocardial extraction of FFA was 0.0215 mEq per 100 ml plasma with a range of from 0.006 to 0.0348 mEq per 100 ml plasma; the mean oxygen extraction ratio was 57.2 per cent ranging from 18 to 79 per cent. Gordon and co-workers found similar values for FFA in their studies on humans (3, 11). The iodine number of TFA was determined in five patients; the coronary sinus levels were consistently higher than arterial levels. The figures in arterial blood as represented in Table II agree with those of Caren and Corbo in venous blood of normal individuals and patients with coronary artery disease (13).

In the studies performed upon fasting dogs (Table III) mean values for arterial levels, myocardial extractions, and oxygen extraction ratios of TFA were 0.956 mEq per 100 ml plasma, 0.042 mEq per 100 ml, and 123 per cent, respectively (range, 0.366 to 1.710 mEq per 100 ml plasma; 0.007 to 0.200 mEq per 100 ml; and 27 to 373 per cent). The arterial FFA concentrations

TABLE III

Results on fasting dogs

Dog no.	N_2O CBF	Myocardial extractions					Myocardial usage		O_2 extraction ratio†	
		Δ O_2*	Art. TFA	Δ TFA	Art. FFA	Δ FFA	TFA	FFA	TFA	FFA
	ml/100 g LV/min	*vol %*	*mEq/100 ml plasma*	*mEq/100 ml plasma*	*mEq/100 ml plasma*	*mEq/100 ml plasma*	*mEq/100 g/min*		%	%
1			0.698	0.0260	0.0584	0.0260				
2	333	5.7	1.152	0.0460	0.0251	0.0042	0.100	0.0091	297	27
3		11.9	1.010	0.0270	0.0215	0.0051			84	15
4	304	8.2	1.210	0.0790	0.0528	0.0057	0.156	0.0113	353	25
5	138	11.6	0.738	0.0160	0.0530	0.0219	0.0141	0.0193	50	68
6	187	7.6	0.990	0.0310	0.0378	0.0045	0.0446	0.0065	179	25
7	88	13.2	0.765	0.0100	0.0366	0.0094	0.0056	0.0053	27	25
8	222	14.0	0.568	0.0126	0.0405	0.0081	0.0182	0.0117	33	21
9	62	5.6	0.752	0.0177	0.0200	0.0026	0.0072	0.0011	118	17
10	60	6.9	0.875	0.0270	0.0274	0.0020	0.0090	0.0006	123	9
11	92	13.2	0.579	0.0154	0.0189	0.0092	0.0079	0.0047	36	21
12	85	16.2	0.865	0.0177	0.0336	0.0112	0.0099	0.0060	38	24
13	92	15.9	0.814	0.0295	0.0299	0.0102	0.0168	0.0058	65	22
14	88	12.2	0.366	0.0142	0.0243	0.0103	0.0082	0.0059	43	21
15	101	15.1	1.492	0.0212	0.0274	0.0140	0.0124	0.0082	46	30
16	87	14.0			0.0364	0.0006		0.0004		1
17	155	6.9	0.848	0.0280	0.0226	0.0096	0.0239	0.0082	125	43
18	124	14.0			0.0270	0.0126		0.0102		33
19	57	16.0	1.049	0.0240	0.0279	0.0105	0.0089	0.0039	55	23
20	93	11.3								
21	89	12.8	1.132	0.0630	0.1177	0.0095	0.0395	0.0060	195	29
22	90	13.2	1.070	0.0120	0.0332	0.0140	0.0071	0.0082	33	39
23	103	14.5	0.750	0.0980	0.0133	0.0076	0.059	0.0046	223	17
24	247	13.5	1.805	0.0900	0.0403	0.0203	0.129	0.0291	220	49
25	105	17.6	1.035	0.0680	0.0200	0.0033	0.0395	0.0019	121	5
26	185	8.9	0.787	0.049	0.0374	0.0098	0.0564	0.0113	193	38
27	174	10.8	0.666	0.016	0.0176	0.0113	0.0162	0.0114	48	34
28	160	12.0	1.174	0.059	0.0223	0.0068	0.0620	0.0072	183	21
29		5.4	0.634	0.007						
30	82	11.7	0.864	0.117	0.0164	0.0031	0.0637	0.00169	373	9
31	49	14.3	1.710	0.031	0.0265	0.0118	0.0085	0.00324	67	25
32	86	14.9	1.290	0.200	0.0189	0.0092	0.1015	0.00467	44	20
33	74	13.5	1.000	0.024	0.0364	0.0164	0.0107	0.00728	60	41
34	71	10.1								
Mean	125	11.9	0.956	0.0420	0.0329	0.0097	0.0384	0.0074	123	26

* Δ Myocardial extraction, i.e., coronary sinus arteriovenous difference.
† See Footnote 1 in text.

TABLE IV

Effect of heparin on dogs

Dog no.	N_2O CBF	Myocardial extractions					Myocardial usage		O_2 extraction ratio†	
		Δ O_2*	Art. TFA	Δ TFA	Art. FFA	Δ FFA	TFA	FFA	TFA	FFA
	ml/100 g LV/min	*vol %*	*mEq/ 100 ml plasma*	*mEq/ 100 ml plasma*	*mEq/ 100 ml plasma*	*mEq/ 100 ml plasma*	*mEq/100 g/min*		*%*	*%*
Fasting dogs										
14‡	88	12.2	0.3658	0.0142	0.0243	0.0103	0.00816	0.0059	43	31
§			0.3469	0.0141	0.0400	0.0108	0.00810	0.0062	42	32
17‡	155	6.9	0.8480	0.0280	0.0226	0.0096	0.02387	0.0082	127	43
§			0.8440	0.0160	0.0425	0.0058	0.01364	0.0050	72	26
23‡	103	14.5	0.7500	0.0980	0.0133	0.0076	0.05882	0.0046	223	17
§			0.7400	−0.0240	0.0244	0.0132	−0.01441	0.0079	−54	30
24‡	247	13.5	1.8050	0.0900	0.0403	0.0203	0.12893	0.0291	219	49
§			1.7390	0.0440	0.0380	0.0185	0.06303	0.0265	107	45
25‡	105	17.6	1.0350	0.0680	0.0200	0.0033	0.03949	0.0019	121	5
§			0.9540	0.0240	0.0445	0.0309	0.01394	0.0180	42	55
26‡	185	8.9	0.7870	0.0490	0.0374	0.0098	0.05639	0.0113	193	38
§			0.7870	0.0110	0.0367	0.0125	0.01266	0.0144	42	49
27‡	174	10.8	0.6660	0.0160	0.0176	0.0113	0.01619	0.0114	48	34
§			0.5670	0.0230	0.0147	0.0053	0.02328	0.0054	70	16
28‡	160	12.0	1.1740	0.0590	0.0223	0.0068	0.06230	0.0072	183	21
§			0.9990	0.1340	0.0205	0.0074	0.14150	0.0078	416	23
Lipemic dogs										
35‡	55	12.3			0.0735	−0.0021		0.00074		6
§	58	11.9			0.0880	0.0190		0.00727		59
36‡	67	13.4			0.0311	0.0111		0.00450		28
§	68	11.5			0.0311	0.0054		0.00256		18
37‡	92	11.5			0.0576	0.0217		0.01358		72
§	82	14.2			0.0227	0.0136		0.00691		33
38‡	50	15.2			0.0266	0.0134		0.00389		29
§	63	16.0			0.0312	0.0161		0.00588		33
39‡	96	16.6			0.0422	0.0222		0.01215		43
§	97	14.4			0.0395	0.0153		0.00980		39
40‡	79	15.2			0.0440	0.0084		0.00385		18
§	47	15.5			0.0507	0.0310		0.00918		71
41‡	65	16.4			0.0843	0.0343		0.01315		70
§	81	17.5			0.0460	0.0032		0.00151		6
42‡	138	14.1			0.0555	0.0243		0.01710		49
Mean of‡	152	12.0	0.9288	0.0527	0.02473	0.0098		0.0099	145	30
fasting dogs§			0.8721	0.0302	0.0326	0.0130		0.0114	92	34
Mean of‡	80.25	14.3			0.0518	0.0166		0.0086		39
lipemic dogs§	73.59	14.4			0.0445	0.0160		0.0068		39

* Δ Myocardial extraction, i.e., coronary sinus arteriovenous difference.
† See Footnote 1 in text.
‡ Before heparin.
§ After heparin.

ranged from 0.0133 to 0.1177 mEq per 100 ml plasma and the myocardial extractions from 0.0006 to 0.0260 mEq per 100 ml plasma. The oxygen extraction ratio of FFA varied from 1 to 68 per cent. In the average dog the extraction of FFA by the heart accounted for only 23 per cent of the total fatty acid extraction.

The effect of alimentary fat and of heparin. Three to four hours after ingestion of a fat meal (Table II), the mean arterial levels of TFA and FFA in patients were, respectively, 1.165 and 0.0761 mEq per 100 ml plasma. Plasma arterial levels of TFA varied from 1.090 to 1.304 mEq per 100 ml plasma, while FFA plasma levels ranged from 0.0668 to 0.0835 mEq per 100 ml plasma. The mean myocardial extraction of TFA was 0.068 and of FFA 0.0201 mEq per 100 ml plasma. These values are comparable with those obtained in fasting patients. The oxygen extraction ratios were 264 and 65 per cent, respectively.

The effect of heparin on fatty acid metabolism

was studied in a limited number of human subjects (Table II). In three individuals, heparin produced a slight fall in the average arterial concentration of TFA from 1.165 to 1.061 mEq per 100 ml plasma and resulted in a marked increase in the arterial concentration of FFA (from 0.0761 to 0.1195 mEq per 100 ml plasma); the myocardial extraction of FFA increased from 0.0201 to 0.0225 mEq per 100 ml plasma. In fasting dogs, similar changes were found 20 minutes after heparin injections. The arterial TFA concentration decreased from a mean of 0.9288 to 0.8721 mEq per 100 ml plasma, that of FFA rose from 0.0247 to 0.0326 mEq per 100 ml plasma. Myocardial extraction of TFA fell from 0.0527 to 0.0302 mEq per 100 ml plasma, while that of FFA rose from 0.0098 to 0.0130 mEq per 100 ml plasma (Table IV). Comparing results obtained on fasting dogs with another group in which Lipomul was given, it may be seen that the fat meal resulted in an elevation of the arterial FFA concentration of from 0.0247 to 0.0518 mEq per 100 ml plasma. The myocardial extraction of FFA also was higher (0.0098 to 0.0166 mEq per 100 ml plasma). The usage of FFA by the heart rose slightly (from 0.0074 to 0.0086 mEq per 100 g per left ventricle per minute; (Tables III and IV). TFA levels were not measured in these dogs, but all had lipemic plasma, and in all the plasma optical densities were higher than any found in fasting animals. In the seven lipemic dogs studied following heparin administration, the arterial levels of FFA rose in three, fell in three, and remained constant in one animal (Table IV). Here, too, the myocardial extraction and usage of FFA varied with their arterial concentration. The TFA concentration in fasting dogs declined slightly with heparin in seven out of eight animals (from a mean of 0.9288 to 0.8721 mEq per 100 ml plasma); however, there was a marked decrease in myocardial extraction (mean of 0.0527 to 0.0302 mEq per 100 ml plasma) and usage of TFA (mean of 0.0492 to 0.0327 mEq per 100 g per left ventricle per minute) in most dogs. In contrast to results obtained on patients with artificially induced hyperlipemia in whom the injection of heparin resulted in a rise in coronary blood flow (14), heparin did not alter the coronary flow in seven hyperlipemic dogs (Table IV).

DISCUSSION

The data on myocardial metabolism of fatty acids by fasting man and dog reveal that FFA average 6 per cent of the mean arterial level of TFA in the human, while in the dog this figure is approximately 3 per cent. In the fasting human the mean myocardial extraction of FFA accounts for 42 per cent of the TFA extraction while the esterified fraction makes up the other 58 per cent. In the fasting dog, the FFA fraction accounts for only 23 per cent of the total fatty acids extracted. The high oxygen extraction ratios for fatty acids confirm previously published experiments in which it could be shown that in the fasting state most of the energy in the heart is derived from fat metabolism (1).

Dole, as well as Gordon and Cherkes have developed the concept that FFA are the blood lipid fraction primarily concerned with the supply of fats to tissues for oxidative metabolism (2, 3). The concentration of the circulating FFA is in some way related to the nutritional state of the subject. If the energy requirements are satisfied by carbohydrates, the FFA level drops to a low value and the myocardial extraction of FFA decreases (3, 11). However, in the fasting state the heart derives only a small part of the energy from carbohydrate substances (15).

Gordon and Cherkes as well as Olson have calculated that the heart can extract fairly large amounts of FFA (3, 15). The results reported here confirm this ability but demonstrate that even in the fasting state, FFA account for less than half of the myocardial extraction of TFA. The likelihood exists that a large fraction of TFA removed by the heart is not immediately oxidized to CO_2 and water. Two of the four fasting patients (Table II) and 13 of 28 dogs in the postabsorptive state (Table III) show oxygen extraction ratios above 100 per cent.

Large myocardial extraction of esterified fatty acids is in line with the finding that over half of the chylomicron triglycerides are directly oxidized (16). These substances may be removed directly by the same tissue sites oxidizing plasma FFA, or lipoprotein lipase present within or on the surface of the myocardial cell may hydrolyze the triglycerides at the site of their oxidation (17). It is unlikely that phospholipids or cholesterol

esters are metabolized by the heart (17). Recently published studies have pointed out the great importance of the plasma FFA fraction in providing readily available foodstuffs to meet metabolic demands throughout the body. The results reported here have demonstrated that the esterified fatty acids account for most of the total fats extracted by the myocardium.

It has been demonstrated that the availability of glucose to the adipose tissue controls the release of FFA into the blood. With depletion of carbohydrate and greater utilization of fat for energy, triglycerides in adipose tissue are hydrolyzed and yield fatty acids to be discharged as FFA into the blood stream (18). Gordon, Cherkes and Gates have found that the myocardial oxygen extraction ratio of FFA decreases from a fasting level of 42 to 0 per cent after an infusion of glucose and insulin (11). The studies reported here were performed either on fasting subjects or after a meal containing only fat. Consequently, a wide range of arterial glucose levels was not present in these patients and animals and the effect of glucose on myocardial utilization of fatty acids could not be ascertained (Tables II and III).

In eight dogs which received a fatty meal, the mean arterial FFA concentration was 0.0518 mEq per 100 ml plasma, while in 34 fasting dogs it was 0.0329 mEq per 100 ml plasma (Tables III and IV). Concomitantly, there was an increase in their myocardial extraction, but the usage of FFA by the heart rose slightly (0.0086 as compared with 0.0074 mEq per 100 ml plasma). This finding illustrates that in the experiments of this series ingestion of enough fat to cause lipemia with a marked increase in optical density of blood increased FFA concentration in plasma. This is in contrast to the findings of Dole, and Gordon and Cherkes, who reported that ingestion of fats to cause lipemia had no effect on FFA concentration in plasma (2, 3). Apparently, as Fredrickson and Gordon have stated, changes in FFA concentration depend upon many factors, including the rate of absorption, rate of transfer of triglycerides from the blood, rate of hydrolysis of triglycerides, rate of utilization of FFA, and the responsiveness of adipose tissue to exogenous fat (17).

Heparin, in the three patients in whom data are available, results in a decline in the arterial concentration of TFA, and in a rise in the concentration of FFA. There is a slight increase in myocardial utilization and extraction of FFA (Table II). In eight fasting dogs, heparin had a similar effect on FFA blood levels and the myocardial extraction and usages increased in most instances concomitantly with the blood levels (Table IV). The effect of heparin in increasing the FFA blood levels of fasting man and dog is in agreement with results of previous investigators (17, 18). Following injection of heparin into seven lipemic animals the arterial levels of FFA rose in three, fell in three, and remained constant in one animal (Table IV). These fluctuations may be the result of changes in blood concentration of fats due to varying rates of absorption. As was found previously, the myocardial extraction and usage of FFA vary with their arterial concentrations. After heparin, the TFA concentration in the blood plasma of fasting dogs declines slightly; however, there is a marked decrease in myocardial extraction and usage of TFA in most of these animals. The finding of a relationship between plasma concentrations and myocardial usage and extraction of FFA is consistent with either active transport or passive diffusion of fatty acids into the cell.

It had been previously found that the injection of heparin into patients with induced hyperlipemia results in an increase in coronary flow (14). In the experiments reported here, heparin had no influence on the coronary flow of hyperlipemic dogs. It is possible that this discrepancy is the result of species differences.

Iodine numbers of the isolated TFA fraction of the plasma were obtained in five patients. The values in coronary sinus blood consistently exceeded those in arterial blood. This suggests that the relative concentration of saturated fatty acids is less in coronary sinus than in arterial blood as a result of greater myocardial usage of saturated fatty acids. Further identification of these saturated fatty acids must await the results of more refined analytical techniques.

SUMMARY

The myocardial extraction and usage of total and free fatty acids (FFA) have been determined in patients and in dogs.

In the postabsorptive state significant amounts of both esterified and free fatty acids were extracted by the myocardium.

The determination of the iodine numbers in plasma revealed that there was proportionately greater usage of saturated than of unsaturated fatty acids by the heart.

The ingestion of a fat meal increased both plasma concentration and myocardial extraction of FFA.

Heparin induced in man and in fasting dogs a fall in the plasma concentration of total fatty acids and an increase in the concentration of FFA.

These studies demonstrate that esterified fatty acids account for more than half of the total fats extracted by the myocardium. Under the experimental conditions reported here, the myocardial extraction of free fatty acids in given patients and animals is usually dependent upon their respective arterial concentrations.

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THE RESPIRATORY RESPONSE TO CARBON DIOXIDE IN HEALTH AND IN EMPHYSEMA

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The tension of carbon dioxide in the cells of the medullary respiratory center stimulates it to transmit efferent nervous impulses to the muscles of respiration. These, in turn, perform mechanical work upon the lung and thorax, and result in the complex movement of air known as ventilation. Ideally, therefore, the stimulus (tissue carbon dioxide tension) and the response (efferent impulses) should be measured in order to assess the response of the respiratory center to carbon dioxide.

Arterial carbon dioxide tension is generally considered to be a satisfactory approximation of the stimulus delivered to the respiratory center, but the response is far more difficult to measure. While most investigations have reported the response in terms of ventilation (1–3), this may be modified by the ventilatory capacity and the degree of airway obstruction (4). The response of the respiratory muscles, therefore, should be a more accurate measure of respiratory center activity. This can be assessed by measuring the total amount of mechanical work done by these muscles.

There is no known method of directly measuring the total mechanical work done by the respiratory muscles. However, if the oxygen consumption (or energy cost) of the respiratory muscles and the efficiency with which the muscles utilize the oxygen are known, the total mechanical work of breathing may be calculated (5, 6).

The purpose of this paper is to report the respiratory response to carbon dioxide, in terms of the total mechanical work performed by the respiratory muscles, in normal subjects and in patients with chronic pulmonary emphysema.

METHOD

Studies were performed on 12 normal subjects and 10 patients with emphysema, whose physical characteristics and pulmonary function assessment are shown in Table I. Vital capacities, expiratory flow rates and maximum breathing capacities were measured on a 9 L Collins respirometer, with the valves and carbon dioxide absorber removed. The maximum of at least 3 determinations was chosen.

All normal subjects had excellent ventilatory function and showed no clinical or radiological evidence of cardiopulmonary disease.

The diagnosis of emphysema was based on the clinical history and physical examination, as well as on radiological and spirographic evidence. The selection of the patients studied was dependent upon their ability to tolerate the prolonged period of inactivity and the severe respiratory effort associated with breathing through an added inspiratory resistance. All patients with emphysema showed marked obstruction to expiratory air flow and reduction of the maximum breathing capacity. Hypoxia was present in all, and carbon dioxide retention in five, while breathing room air at rest. On the other hand, hypercapnia was present in all when breathing 100 per cent oxygen. In addition, the oxygen cost of an increase in ventilation was high, and the efficiency of the respiratory muscles was low.

The subjects were studied in the postabsorptive state, and rested in a comfortable easy chair for one hour before any measurements were made. An indwelling Riley needle was inserted into the brachial artery of the patients with emphysema. All subjects then breathed to and from a closed-circuit spirometer, through varying lengths of dead space for at least 20 minutes. During the last 7 to 10 minutes, records of ventilation and oxygen consumption were obtained. A rest period of 10 to 15 minutes was allowed between measurements.

Oxygen consumption and ventilation were measured by a method previously described (5–7). Increased levels of ventilation were achieved by interposing dead space consisting of thick-walled rubber tubing (3 cm ID) between the spirometer and the subject. Minute ventilation was expressed in liters per minute BTPS and oxygen consumption in milliliters per minute STPD.

An end-tidal sampler, modified from Rahn and Otis (8), was incorporated into the breathing valve, and end-tidal air was analyzed for carbon dioxide, by means of a rapid infrared Liston-Becker gas analyzer. The end-tidal carbon dioxide tension, used as an index of arterial carbon dioxide in the normal subjects,[1] was determined

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[1] Hereafter, for the sake of simplicity, all measurements of carbon dioxide tension will be referred to as arterial carbon dioxide tension.

TABLE I

Physical characteristics and pulmonary function of normal and emphysematous subjects *

Subject no. and sex	Age	Ht.	BSA	Vital capacity		Maximum breathing capacity		Arterial pCO_2			Efficiency	O_2 cost	Resting work
					% Pred.		% Pred.	Arterial pO_2	Room air	100% O_2			
	yrs	*cm*	m^2	*L*		*L/min*		*mm Hg*			%	*ml/L*	*kg-m/min/m²*
1 F	22	178	1.83	4.42	125	131	122			38	10.0	0.9	0.8
2 M	21	189	2.05	5.94	124	205	140			39	10.2	2.7	2.6
3 F	19	165	1.72	3.84	116	125	125			39	10.9	0.6	0.7
4 M	25	179	1.99	5.17	118	220	166			40	5.9	1.3	0.6
5 M	26	183	1.93	5.45	121	173	128			41	9.1	1.1	1.0
6 M	43	170	1.87	4.76	125	186	181			41	6.5	0.9	0.6
7 M	24	178	2.0	6.20	162	192	160			41	7.9	0.7	0.3
8 M	29	193	2.21	6.88	143	160	115			42	6.5	1.1	0.6
9 M	25	173	1.79	4.98	120	182	145			42	8.1	1.3	1.2
10 M	34	180	1.90	6.20	152	182	152			42	9.3	2.5	1.7
11 M	26	182	1.98	5.50	125	143	108			43	8.0	2.0	2.1
12 M	22	178	1.90	5.40	121	286	214			46	7.9	0.5	0.4
13 M	57	180	1.97	2.78	71	49	48	47	38	50	1.6	3.2	0.5
14 M	69	180	1.76	2.93	80	42	47	52	40	70	2.1	2.8	0.8
15 M	46	169	1.68	2.34	64	27	26	57	41	47	4.3	3.3	1.5
16 M	61	179	1.78	2.73	71	18	18	68	41	51	2.0	3.9	1.3
17 M	63	173	1.68	1.69	48	17	19	49	43	57	1.5	9.1	1.9
18 M	60	175	1.65	1.83	49	11	12	48	51	51	0.7	14.2	1.1
19 M	73	155	1.31	1.64	60	20	40	58	55	59	1.0	8.4	0.9
20 M	53	173	1.70	2.05	57	20	21	62	56	57	1.9	4.9	1.2
21 M	69	179	1.86	1.64	47	27	31	36	60	63	2.2	6.1	1.2
22 M	43	168	1.72	3.64	98	28	27	69	61	61	2.1	4.2	1.5

* Subjects 1 through 12 are normal; Subjects 13 through 22 are emphysematous.

for 1 to 2 minutes immediately before and after the recording period, so that for all calculations, no allowance had to be made for the air passing through the analyzer. No difference was noted between the readings taken before and after the recording period.

An arterial blood sample was drawn from the emphysematous subjects before beginning the experiment while they were breathing ambient air, and over the fourth to seventh minutes of each recording period. Arterial gas tensions were measured by the method of Riley, Proemmel and Franke (9), as modified by Brinkman, Johns, Donoso and Riley (10).

Measurements of ventilation, oxygen consumption and pCO_2 were made at rest and at three levels of increased ventilation, except in Subjects 6, 17 and 19. In these three patients, measurements were made at two levels of increased ventilation.

The oxygen cost of increased ventilation was calculated by plotting oxygen consumption against minute ventilation, and visually drawing the best-fitting straight line. The slope of this line, in milliliters per liter expressed the oxygen cost of increased ventilation. The oxygen consumption of the respiratory muscles at any level of ventilation could then be calculated by multiplying the oxygen cost by the observed ventilation.

The "efficiency" of the respiratory muscles was obtained by measuring the increase in oxygen consumption associated with an added respiratory work load of known magnitude, as previously described (5). The increase in respiratory work was achieved by having the subject inspire through a wide-bore metal pipe, projecting under a water seal. There was no change in resting level while breathing through the added resistance in either the normal or emphysematous subjects. In the latter, there was a slight increase in ventilation, however. This necessitated comparison with the oxygen consumption at an equivalent ventilation. This could be derived from the line relating ventilation and oxygen consumption when there was no added work load. Since the oxygen consumption before, and while breathing through the water seal, were compared at equivalent ventilations, and there was no change in resting level, the efficiency of the respiratory muscles for handling added work could be calculated.

Since the efficiency of the respiratory apparatus has been shown to be essentially the same at various levels of ventilation (5–7), the total mechanical work of breathing could be calculated at any level of ventilation. This was done by multiplying the observed efficiency by the mechanical energy equivalent of the oxygen consumption of the respiratory apparatus, and was expressed in kilogram-meters per minute.

RESULTS

Figure 1 presents the effect of changes in arterial carbon dioxide tension on the minute ventilation in the normal and emphysematous sub-

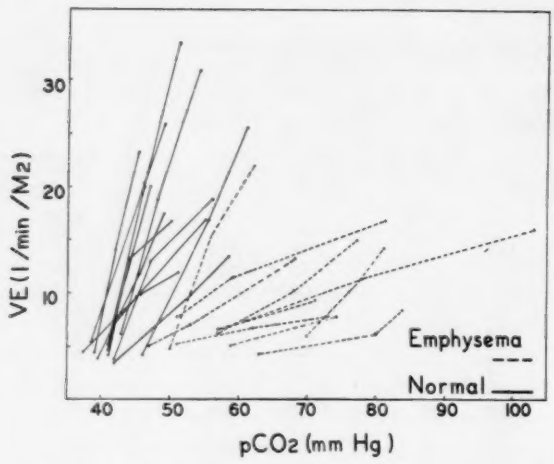

FIG. 1. THE EFFECT OF CHANGES IN ARTERIAL pCO_2 ON THE MINUTE VENTILATION IN 12 NORMAL SUBJECTS AND 10 PATIENTS WITH PULMONARY EMPHYSEMA.

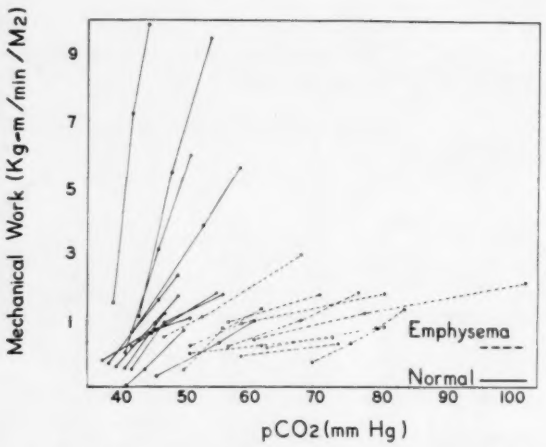

FIG. 3. THE EFFECT OF CHANGES IN ARTERIAL pCO_2 ON THE TOTAL MECHANICAL WORK OF BREATHING IN 12 NORMAL SUBJECTS AND 10 PATIENTS WITH PULMONARY EMPHYSEMA.

jects. It can be noted that although there was a wide range of slopes of ventilatory response in the normal group, the mean being 1.70 L per mm Hg pCO_2 (range 0.59 to 3.18), the emphysematous subjects tended to have a markedly diminished ventilatory response to carbon dioxide, the mean slope being 0.77 L per mm Hg pCO_2 (range 0.11 to 3.57).

The effect of changes in arterial carbon dioxide tension on the oxygen consumption in the normal and emphysematous subjects is presented in Figure 2. It can be seen that the slopes of the oxygen consumption lines tended to be similar in the normal and emphysematous subjects. The mean slope was 2.33 ml per mm Hg pCO_2 (range 0.52 to 8.69) in the normals and 1.79 ml per mm Hg (range 0.87 to 4.84) in the patients with emphysema.

In Figure 3, the effects of changes in arterial carbon dioxide tension on the total mechanical work of breathing are shown. It can be seen that although there was some similarity between the slopes of the response lines of mechanical work of breathing in the two groups, the mechanical work response to an increase in arterial carbon

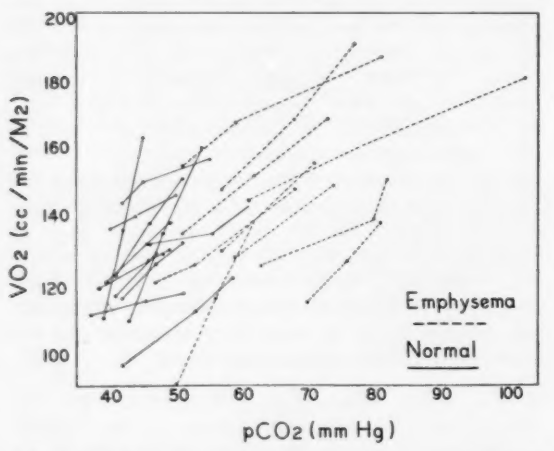

FIG. 2. THE EFFECT OF CHANGES IN ARTERIAL pCO_2 ON THE OXYGEN CONSUMPTION IN 12 NORMAL SUBJECTS AND 10 PATIENTS WITH PULMONARY EMPHYSEMA.

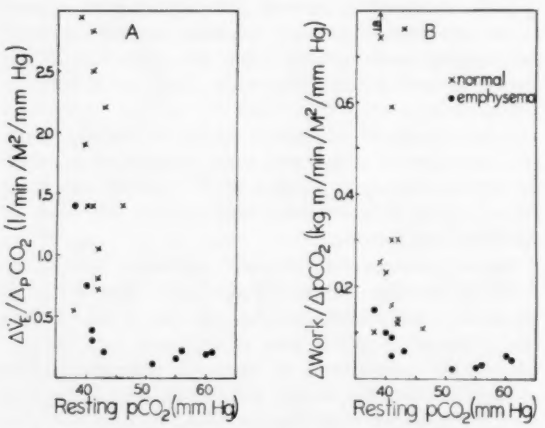

FIG. 4. THE RELATIONSHIP BETWEEN RESTING pCO_2 WHILE BREATHING AIR, AND THE SLOPES OF THE VENTILATORY RESPONSE, A, AND THE TOTAL MECHANICAL WORK RESPONSE, B, TO CARBON DIOXIDE IN NORMAL AND EMPHYSEMATOUS SUBJECTS.

TABLE II

The effect of artificial airway obstruction on ventilatory function

Subject	pCO_2		Ventilation		Maximum breathing capacity		Efficiency		Oxygen cost		Resting mechanical work	
	N*	Obs.†	N	Obs.	N	Obs.	N	Obs.	N	Obs.	N	Obs.
	mm Hg		*L/min/m²*		*L/min*		%	%	*ml/L*		*kg-m/min/m²*	
4	40	41	3.9	4.1	220	77	5.9	4.8	1.3	4.1	0.6	1.9
5	41	39	5.1	4.3	173	58	9.1	5.2	1.1	2.8	1.0	1.6
6	41	39	5.1	5.3	186	67	6.5	2.4	0.9	2.8	0.6	0.8
12	46	44	4.4	4.4	286	104	7.9	3.6	0.5	2.7	0.4	1.0
Mean	42	40.8	4.6	4.5	216	75	7.4	4.0	1.0	3.1	0.7	1.3

* Resting breathing.
† Obstructed breathing.

dioxide tension was lower in most of the emphysematous subjects. The mean slope was 0.42 kg-m per minute per m^2 per mm Hg pCO_2 (range 0.11 to 1.84) in the normals, and 0.07 kg-m per minute per m^2 per mm Hg pCO_2 (range 0.02 to 0.17) in the patients with emphysema.

The relationship between the resting arterial pCO_2 while breathing ambient air and the slopes of the stimulus response lines of the normal and emphysematous subjects is shown in Figure 4. The slopes of the ventilatory response are plotted in A, while the slopes of the total mechanical work response are plotted in B. It can be seen that the slopes of both the ventilatory and total mechanical work response lines tended to be diminished in the patients with emphysema, particularly in those who were hypercapnic at rest.

Since it has been shown that airway resistance will reduce the ventilatory response to carbon dioxide (4), it was desirable to ascertain the effect of obstruction on the oxygen consumption and mechanical work of breathing response to changes in arterial carbon dioxide. The entire procedure was therefore repeated with four normal subjects, at a different sitting, while breathing through an artificial airway obstruction. The obstruction consisted of a metal pipe (3 cm ID), which was packed with glass wool, and which offered a resistance of 0.3 cm H_2O per L per second at flow rates between 10 and 100 L per second.

Table II shows the effect of the artificial airway obstruction on pulmonary function in the four normal subjects. It can be seen that there was no change in resting arterial carbon dioxide tension or minute ventilation while breathing through the obstruction. On the other hand, it can also be seen that the maximum breathing capacity and the calculated efficiency of the respiratory apparatus were reduced, while the resting total mechanical work of breathing and the oxygen cost of increased ventilation rose markedly.

The influence of airway obstruction on the

TABLE III

The effect of artificial airway obstruction on respiratory response to carbon dioxide

Subject	$\Delta VE/\Delta pCO_2$*		$\Delta VO_2/\Delta pCO_2$†		Δ Work/ΔpCO_2‡	
	N§	Obs.‖	N	Obs.	N	Obs.
	L/min/m²/mm Hg		*ml/min/m²/mm Hg*		*kg-m/min/m²/mm Hg*	
4	1.37	0.51	1.76	2.02	0.23	0.22
5	2.90	1.72	2.78	5.20	0.59	0.52
6	2.48	1.41	2.08	3.84	0.27	0.20
12	1.37	0.58	0.53	1.53	0.11	0.12
Mean	2.03	1.06	1.79	3.12	0.30	0.27

* Change in ventilation per mm Hg pCO_2.
† Change in oxygen consumption per mm Hg pCO_2.
‡ Change in mechanical work per mm Hg pCO_2.
§ Resting breathing.
‖ Obstructed breathing.

respiratory responses to carbon dioxide is shown in Table III. It can be seen that the slope of the ventilatory response to carbon dioxide was sharply reduced by an airway obstruction, while the slope of the oxygen consumption response to carbon dioxide was increased. On the other hand, the slope of the total mechanical work response to carbon dioxide was not altered by the introduction of an airway obstruction.

DISCUSSION

It is apparent that the conclusions which can be drawn from this paper are dependent upon the interpretation of the data which have been presented. Thus, if increments in ventilation or mechanical work are accepted as the response of the respiratory system to changes in carbon dioxide, it can be concluded that the sensitivity of the respiratory system was diminished in patients with emphysema. On the other hand, if increments in oxygen consumption are regarded as the response of the respiratory system, it can be concluded that there was no alteration in sensitivity of the respiratory system in the emphysematous subjects. For reasons outlined below, we are in favor of the theory that the response of the respiratory system is best reflected by the increments in total mechanical work done by the respiratory muscles.

In any study of the response of the respiratory system to carbon dioxide, consideration should be given to both the minimum stimulus necessary to evoke a response, and the slope of the stimulus-response line. No measurements of threshold stimulus were made in this study. Nevertheless, the finding of hypercapnia in the presence of a normal resting minute ventilation and total mechanical work of breathing, suggests that the threshold of response was altered in the patients with emphysema.

The finding that the administration of oxygen led to hypercapnia even in those patients who were normocapnic at rest while breathing room air is surprising and difficult to explain. Richards, Fritts and Davis (11) have shown that while the arterial pCO_2 rose in patients with emphysema who were breathing oxygen-enriched gas mixtures, the slope of the stimulus-response line of any one subject was unaltered. In the present paper, the ventilatory and total mechanical work response lines were diminished from the normal in almost all of the emphysematous subjects, particularly those who were hypercapnic at rest even while breathing room air. This suggests that the sensitivity of the respiratory system was diminished.

The fact that both a diminished ventilatory and total mechanical work response to carbon dioxide were found in patients with emphysema, suggests no advantage to the measurement of the total mechanical work response as an index of the sensitivity of the respiratory system. However, the data presented in this paper, and those of Cherniack and Snidal (4), indicate that the slope of the ventilatory response to carbon dioxide can be altered by breathing through an artificial airway resistance. Moreover, the ventilatory response to carbon dioxide can be increased after the administration of nebulized bronchodilator in emphysematous patients (4). Unless the respiratory center sensitivity was changed acutely in these subjects, these findings suggest that increments in ventilation are an inadequate expression of respiratory center response.

The data presented also indicate that the change in oxygen uptake, in response to a rise in arterial carbon dioxide tension, is similar in normal and emphysematous subjects. This is in agreement with the data of Richards and associates (11), whose interpretation was that the response of the respiratory center and the muscles of patients with emphysema was essentially normal. However, the amount of oxygen consumed by the respiratory muscles for a given unit of work done will depend upon the efficiency of the respiratory muscles. Thus, when comparing the response of the respiratory system in two groups of individuals, the increments in oxygen consumption may be compared only when the efficiency of the respiratory muscles is similar in the two groups being compared. It is important to differentiate between energy cost and energy output in any mechanical or biological system, where efficiencies may vary. The data presented in this paper indicate that the efficiency of the respiratory muscles was considerably lower than normal in the patients with emphysema. Thus, the work output of the respiratory apparatus in response to changes in CO_2 was considerably lower than normal in these patients.

In addition, the data presented in this paper indicate that the slopes of ventilation and oxygen consumption increments in response to carbon dioxide could be altered while breathing through an artificial airway obstruction, while the slope of the total mechanical work response was unaltered. Assuming no acute change in respiratory center sensitivity while breathing through the obstruction, it may be presumed that the center will respond in the same way to changes in carbon dioxide whether or not obstruction is present. However, because of the obstruction, the response, in terms of ventilation and the oxygen cost of this ventilation, will be altered. It is therefore proposed that incremental changes in total mechanical work are a more accurate gauge of the response of the respiratory apparatus to carbon dioxide than are increments of ventilation or oxygen consumption.

It should be pointed out that while resting pCO_2 and ventilation were unchanged during obstructed breathing, the total mechanical work at rest was increased. If total mechanical work is a better gauge of the activity of the respiratory system, this suggests that a given stimulus was producing a greater response during resting obstructed breathing. Thus, this would represent an increase in activity of the respiratory center when the subject was breathing through an obstruction.

The conclusions reached in this paper are dependent on certain assumptions which are inherent in the methods of determining the oxygen cost, and the efficiency, of the muscles of respiration. It is assumed that the efficiency with which the respiratory apparatus handles added work loads is similar to its efficiency without added work loads, at rest or at low levels of ventilation, and during isometric contraction. The interpretation of the data presented is valid, therefore, only within the limits of these assumptions.

SUMMARY AND CONCLUSION

1. The respiratory response to carbon dioxide was assessed in 12 normal and 10 emphysematous subjects.

2. Evidence is presented to suggest that incremental changes in total mechanical work are a more accurate gauge of the respiratory response to carbon dioxide than are increments of ventilation or oxygen consumption.

3. The response of the respiratory center and muscles of patients with emphysema, when measured in terms of total mechanical work done, is lower than that of normal subjects.

4. The response of the emphysematous subjects with carbon dioxide retention at rest while breathing room air, tends to be depressed below that of the patients who were normocapnic.

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STUDIES ON LIPID METABOLISM IN THE SMALL INTESTINE WITH OBSERVATIONS ON THE ROLE OF BILE SALTS * †

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It is now well established that the esterification of long chain fatty acids is an important step in their transport across the small intestinal mucosa before they appear in the lymph in chylomicrons. Evidence for this process has been well reviewed recently (1, 2). The main ester formed by the mucosa is triglyceride but a small proportion of the fatty acids is incorporated into phospholipids and cholesterol esters. We have recently reported some *in vitro* studies on the incorporation of long chain fatty acids into glycerides using homogenates of rat and human small intestine mucosa and have delineated some of the cofactor requirements involved (3). This work has now been extended to the intact mucosa of the rat. Observations are presented on factors influencing the uptake and esterification of palmitate-1-C^{14} and the incorporation of label into lipid from C^{14}-glucose. In addition, evidence is presented for the concept that conjugated bile salts directly stimulate glyceride metabolism in the intestinal mucosa.

MATERIALS AND METHODS

Palmitic acid-1-C^{14} and uniformly labeled glucose-C^{14} were obtained from the Volk Radiochemical Company, Chicago, Illinois. The C^{14}-labeled palmitate was purified and made up into a solution as previously described (2). Crystalline bovine albumin was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. Cholic acid and desoxycholic acid were obtained from Matheson, Coleman and Bell, Norwood, Ohio and the cholic acid was recrystallized from 70 per cent ethanol. The taurine derivatives of these acids and glycocholic acid were prepared according to Norman (4) and their purity tested by melting point determinations and paper chromatography (5). Hydrolysis of the conjugated bile salts, glycocholate and taurodesoxycholate was carried out by dissolving them in 2 N NaOH and then heating in sealed Pyrex tubes at 140° C for 3 hours. The resulting solution was diluted with water, acidified, and the precipitated free bile salt filtered, washed and dried. In earlier experiments commercial sodium taurocholate (Pfanstiehl Laboratory, Waukegan, Ill.) was used. This contained a faint trace of glycocholate which could only be demonstrated by chromatography when there was heavy overloading of Whatman no. 3 filter paper. No unconjugated bile salts were present but there were some contaminating pigments. The commercial taurocholate gave substantially the same results as the synthetic material which was used in later experiments.

Female albino rats (Charles River Laboratories, Boston, Mass.) weighing 150 to 250 g were fasted overnight. They were killed by a blow on the head and the small intestine washed out at room temperature with oxygenated Krebs-Ringer phosphate buffer, pH 7.4, modified to contain half the usual concentration of calcium. This buffer was used in all the experiments described. The small intestine was everted on a glass rod following the technique of Wilson and Wiseman (6) and then cut across so as to form small cylindrical segments or intestinal rings. These measured approximately one-eighth inch (lengthwise) and weighed between 80 to 150 mg (wet weight). Since one of the problems in using slices or sacs of small intestine in metabolic studies is the variation in activity along this organ, some preliminary experiments were performed to determine the distribution of esterifying activity along the intestine. It was found that under optimal conditions there was moderate (50 per cent) variation of activity along the upper four-fifths of the jejuno-ileum. The terminal ileum showed an abrupt and profound fall off in activity to 5 to 10 per cent that of the upper intestine. Colonic tissue was about as active as terminal ileum. Thus the reproducibility of our method was increased in subsequent studies by *1*) using only the upper two-thirds of the jejuno-ileum, *2*) by using at least two segments of intestine in each incubation flask with each segment coming from a different site, and *3*) by performing all incubations in duplicate. Incubations were carried out in 25 ml Erlenmeyer flasks and the incubation mixture contained either palmitate-1-C^{14} or uniformly labeled glucose-C^{14} plus other agents such as bile salts, Tween "80"

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(polyoxyethylene sorbitan mono-oleate) and albumin as indicated in the individual experiments. The incubation mixture was made up to a volume of 4 ml with buffer. All incubations were performed under oxygen at 25° C in a Dubnoff shaking incubator. It was found that incubating at 25° as compared to 37° C reduced the transport of esterified lipid out of the tissue to less than 0.1 per cent [using the everted sac technique (6)] and thus increased the validity of simply analyzing tissue lipid at the end of the incubation. This temperature also permitted a better demonstration of the effects of bile salts on the system. At the end of the incubation the flasks were placed on ice and the intestinal segments washed in cold Krebs-Ringer buffer, blotted and weighed on a microtorsion balance. The method of extracting lipid from the tissue was based on that of Folch, Lees and Sloane Stanley (7). Segments were homogenized in chloroform-methanol (2:1) in a tissue grinder (Potter-Elvehjem, Teflon pestle) and the mixture allowed to stand at least 30 minutes at room temperature. It was then filtered through Whatman no. 1 filter paper into a 50 ml Pyrex centrifuge tube which was fitted with a ground glass stopper. A volume of water was added which was approximately one-fifth of the chloroform-methanol solution. The mixture was shaken and the separation into two phases facilitated by centrifuging at 2,500 rpm for 5 minutes. The upper aqueous methanol phase was drawn off and discarded. In experiments with radioactive palmitate the resulting chloroform solution was evaporated to dryness and the lipid, after being dissolved in 10 ml of petroleum ether, was washed once with 20 ml of 0.1 M KCl to remove any nonlipid radioactive metabolites. The total lipid radioactivity was assayed by counting 2 ml of this petroleum ether extract in a lipid scintillation spectrometer (3). Duplicate tissue analyses agreed within 10 to 15 per cent. Another aliquot of the petroleum ether solution was evaporated to dryness in a lipped test tube, taken up in 2 ml of wet ether and the unesterified fatty acids removed by passage through an Amberlite IRA-400 column. The ether eluate representing esterified lipid was evaporated, taken up in petroleum ether, and counted. In experiments with C^{14}-labeled glucose the chloroform solution containing the lipid was evaporated and the lipid taken up in 10 ml of petroleum ether and washed three times with approximately 0.1 M KCl before counting an aliquot of the petroleum ether extract. Negligible water-soluble radioactivity remained in the petroleum ether under such conditions. In experiments where further fractionation of the lipid was to be performed by silicic acid chromatography, all evaporations were conducted under nitrogen, the samples were not heated to above 37° C and they were stored at −10° C in tubes previously gassed with nitrogen.

Other methods and the preparations of materials have been described previously (3). Tissue from guinea pig, hamster and rabbit were prepared in a manner similar to that used for rat tissue. Human jejunal tissue was obtained at operation from patients undergoing partial gastrectomy and was immediately placed in oxygenated Krebs-Ringer buffer at room temperature and transported as quickly as possible to the laboratory for incubation. This process took less than 20 minutes.

RESULTS

Studies on binding versus esterification of palmitate-1-C^{14}. When a slice of everted rat small intestine is incubated with only palmitate-1-C^{14} and buffer, there is a large uptake of labeled fatty acid by the tissue, but only a negligible proportion of this fatty acid is esterified. The addition of substances to the incubation medium which are known to maintain fatty acids in solution, such as albumin, Tween "80," and taurocholate, decrease the total uptake of palmitate-1-C^{14} but significantly increase the amount which is esterified (Table I). Of these three agents taurocholate is by far the most effective. It seemed likely that the large uptake of radioactivity in the absence of one of these substances represented binding of the fatty acid to the cell surface. This possibility was investigated by examining the cellular distribution of the radioactivity in intestinal slices after an incubation. The results (Table II) indicate that in the absence of a wetting agent (taurocholate) most of the radioactivity was present in the cell debris, which contained cell walls and nuclei. This radioactivity was in the unesterified lipid fraction. These results were not significantly different when the incubation was performed under nitrogen or at 4° rather than at 37° C. On the other hand, when taurocholate was added to

TABLE I

The uptake and esterification of palmitate-1-C^{14} by slices of rat small intestine *

Additions†	Palmitate-1-C^{14}/100 mg tissue		
	Total	Unesterified	Esterified
	mμmoles	*mμmoles*	*mμmoles*
None	7.2	7.0	0.2
Albumin (5 mg/ml)	4.8	4.2	0.6
Tween "80" (0.38 %)	3.3	1.6	1.7
Taurocholate (2×10^{-2} M)	5.7	1.0	4.7

* All flasks contained in addition to substances listed above palmitate-1-C^{14}, 100 mμmoles (5×10^5 cpm), and Krebs-Ringer phosphate buffer to a final volume of 4 ml. The incubation was for 1 hour at 25° C.

† Figures in parentheses represent concentration in the incubation medium.

TABLE II

The localization of palmitate-1-C^{14} in slices of rat small intestine *

Additions	Gas phase	Palmitate-1-C^{14}/100 mg tissue					
		Cellular debris†			Supernatant†		
		Total	Unesterified	Esterified	Total	Unesterified	Esterified
		mμmoles	*mμmoles*	*mμmoles*	*mμmoles*	*mμmoles*	*mμmoles*
None	Oxygen	22.9	22.0	0.9	0.8	0.6	0.2
None	Nitrogen	22.3	21.8	0.5	0.6	0.4	0.2
Taurocholate (2×10^{-2} M)	Oxygen	6.6	1.1	5.5	2.5	0.2	2.3

* All flasks contained palmitate-1-C^{14}, 100 mμmoles (5×10^5 cpm), and Krebs-Ringer phosphate buffer in a final volume of 4 ml. The incubation was for 1 hour at 25° C.

† Based on the centrifugation at 600 × G at 4° C of a 0.15 M KCl homogenate of the slices.

the incubation medium a considerable proportion of the radioactivity was present in the supernatant fraction and was esterified. Additional experiments with albumin and Tween "80" gave results similar to those with taurocholate. As would be expected from our previous studies on palmitate esterification (3), this process in the intestinal slices was energy-dependent. An energy requirement was shown by performing incubations with 2×10^{-2} M taurocholate anaerobically or in the presence of either dinitrophenol (10^{-4} M) or potassium fluoride (2×2^{-2} M). These conditions resulted in inhibition of palmitate esterification (viz., 85, 96 and 64 per cent inhibition, respectively).

The effect of conjugated bile salts on the esterification of palmitate-1-C^{14}. The conjugated bile salts studied were taurocholate, glycocholate and taurodesoxycholate and all stimulated the esterification of palmitate-1-C^{14}. The respective curves demonstrating the effect of increasing concentrations of these salts are shown in Figure 1. The cholate derivatives had an optimal concentration of 1.5 to 2×10^{-2} M while the optimal concentration for taurodesoxycholate was 5×10^{-3} M. Higher concentrations of taurodesoxycholate inhibited the reaction. These curves also show that there was only negligible stimulation by the cholate derivatives below concentrations of 5×10^{-3} M and by taurodesoxycholate below 10^{-3} M. However, the addition of two bile salts (glycocholate and taurodesoxycholate), in concentrations at which each salt alone produced no effect, resulted in stimulation (Table III). The optimal activity of each of these three bile salts studied was comparable under our experimental conditions.

In view of the fact that rat bile contains predominantly taurocholate (8–10) it was of interest to study the effect of this salt on tissue from species which predominantly have glycine-conjugated

TABLE III

The additive effect of different bile salts on the esterification of palmitate-1-C^{14} by slices of rat small intestine *

Bile salt added		Palmitate-1-C^{14} esterified/100 mg tissue
Glycocholate	Taurodesoxycholate	
μmoles/ml	*μmoles/ml*	*mμmoles*
0	0	0.58
5	0	0.64
0	1	0.48
5	1	3.4

* Each flask contained palmitate-1-C^{14}, 100 mμmoles (5×10^5 cpm), and Krebs-Ringer phosphate buffer to give a final volume of 4 ml. The incubation was for 1 hour at 25° C.

TABLE IV

The effect of taurocholate on the esterification of palmitate-1-C^{14} by slices of small intestine of various species *†

Species	Additions	
	None	Taurocholate (2×10^{-2} M)
Guinea pig	3.1	9.2
Hamster	6.1	27.6
Human	2.0	17.3
Rabbit	2.2	5.5
Rat	1.6	3.8

* Results expressed as millimicromoles palmitate-1-C^{14} esterified per 100 mg intestine, except human tissue which is per 100 mg intestinal mucosa.

† Each flask contained palmitate-1-C^{14}, 100 mμmoles (5×10^5 cpm), 20 mg of albumin and Krebs-Ringer phosphate buffer to a final volume of 4 ml. The incubation was for 1 hour at 25° C.

TABLE V

Comparison of the effect of Tween "80" and taurocholate on the esterification of palmitate-1-C¹⁴ by slices of rat small intestine *

Tween "80"	Taurocholate	Palmitate-1-C¹⁴ esterified/ 100 mg tissue
%	*μmoles/ml*	*mμmoles*
0.38	None	1.2
0.5	None	1.1
0.38	20	4.2
None	20	4.6

* Each flask contained palmitate-1-C¹⁴, 100 mμmoles (5 × 10⁵ cpm), and Krebs-Ringer phosphate buffer to give a final volume of 4 ml. The incubation was for 1 hour at 25° C.

bile salts (hamster, rabbit and guinea pig) and from those which excrete a more equal mixture of glycine and taurine conjugates (man) (8–10). In all these species taurocholate (2×10^{-2} M) stimulated the esterification of palmitate-1-C¹⁴ (Table IV).

Since both taurocholate and Tween "80" are good emulsifying agents, the observation in Table I that taurocholate was 2.5 times as effective as Tween "80" in stimulating the esterification of palmitate was further investigated. The results in Table V demonstrate that at optimal taurocholate concentrations the addition of Tween "80" had no effect. In contrast, at optimal Tween "80" concentrations the addition of taurocholate produced a further stimulation (fourfold). The fact that taurocholate was more effective than Tween "80" in stimulating the esterification of palmitate-1-C¹⁴ suggested that taurocholate might have another role besides that of an intraluminal emulsifier of fat. It seemed probable that if taurocholate were merely acting as an emulsifying agent, preincubation of small intestine for 30 minutes with taurocholate, before the addition of palmitate-1-C¹⁴, would offer no advantage over control tubes preincubated with buffer only. However, if taurocholate also had a cellular effect, preincubation with the bile salt might stimulate palmitate esterification. Such experiments were performed with taurocholate, glycocholate and taurodesoxycholate and compared to the action of a wetting agent such as Tween "80". Table VI demonstrates that preincubation with these bile salts resulted in stimulation of palmitate esterification while Tween "80" had no such effect.

The effect of conjugated bile salts on the incorporation of label into lipid from glucose-1-C¹⁴. Further evidence that conjugated bile salts stimulate lipid metabolism in the mucosal cell is afforded by experiments using C¹⁴-labeled glucose. We have observed that when everted rat small intestine is incubated with a tracer amount of uniformly labeled C¹⁴-glucose, a significant proportion of the radioactivity is incorporated into the tissue lipid (Table VII). Following saponification of such lipids a large proportion (i.e., greater than 95 per cent) of the radioactivity is in the water-soluble fraction, presumably in gly-

TABLE VI

The effect of preincubating slices of rat small intestine with conjugated bile salts and Tween "80" upon the esterification of palmitate-1-C¹⁴ *

Addition	No. of Expt.	Palmitate esterification/100 mg tissue after preincubation with: Buffer only — Mean	Buffer only — Range	Buffer plus wetting agent — Mean	Buffer plus wetting agent — Range	Mean change in palmitate esterification
		mμmoles		*mμmoles*		%
Taurocholate (10^{-2} M)	6	1.1	0.7 – 1.5	1.9	1.0 – 2.4	+71
Glycocholate (10^{-2} M)	6	1.2	0.9 – 1.7	1.8	1.6 – 2.6	+45
Taurodesoxycholate (1.5×10^{-3} M)	2	0.18	0.16 – 0.20	0.30	0.28 – 0.32	+66
Tween "80" (0.1 %)	4	0.43	0.28 – 0.60	0.40	0.24 – 0.61	−7

* Control slices were preincubated in Krebs-Ringer phosphate buffer (column 3) and experimental slices with buffer plus bile salt or Tween "80" (column 4). After 30 minutes, palmitate-1-C¹⁴ (100 mμmoles) was added to both, as well as bile salts or Tween "80" to the controls in order to make the final concentration of the wetting agents equal in each set of flasks. Incubation was then carried out for 30 minutes.

TABLE VII

The effect of taurocholate on C^{14}-glucose metabolism by rat small intestine *

Addition	Radioactivity/100 mg tissue	
	Total lipid	Barium-ethanol ppt.
	cpm	*cpm*
None	4,160	3,430
Taurocholate (2×10^{-2} M)	16,400	3,360

* Each flask contained uniformly labeled glucose, 0.02 μmoles (5×10^5) cpm, and Krebs-Ringer phosphate buffer to give a final volume of 4 ml. The incubation was for 1 hour at 25° C. After incubation the tissue was washed, weighed and then homogenized in 0.15 M KCl. One aliquot was used for lipid analysis. To another aliquot 1 μmole of carrier fructose-1,6-diphosphate was added and the phosphorylated carbohydrates precipitated with barium and ethanol (11), plated on aluminum planchets and counted in a gas-flow counter. Correction was made for the different counting efficiency of the liquid scintillation spectrometer and the gas-flow counter. Each result is a mean of triplicate determinations.

cerol. The presence of taurocholate (2×10^{-2} M) in the medium increases the incorporation of radioactivity into the lipid up to fourfold (Table VII). Such an increase in the radioactivity in tissue lipid could possibly result from an increased labeling of the intracellular glucose pool, secondary to a greater transport of the tracer glucose into the cell. If this were the explanation, one would also expect an increase in the labeling of other products of glucose metabolism, such as phosphorylated carbohydrate intermediates. The phosphorylated carbohydrate intermediates were therefore isolated by barium-ethanol precipitation (11) and did not show any increase in radioactivity when taurocholate was present in the incubation medium (Table VII). Glycocholate and taurodesoxycholate also stimulated the incorporation of radioactivity into lipid when rat small intestine was incubated with C^{14}-labeled glucose. The effect of varying concentrations of these salts on this process is shown in Figure 2. A comparison of the two sets of curves in Figures 1 and 2 shows that the optimal concentration for each bile salt was similar for both the labeled palmitate and labeled glucose experiments.

The formation of glycerol-labeled lipid from C^{14}-labeled glucose in our experiments lends support to the hypothesis that glucose is a glyceride-glycerol precursor in the mucosal cell during fatty acid absorption (12, 13). This was further substantiated by observing a twofold increase of label in tissue lipid when 1 μmole of unlabeled palmitate was added to the C^{14}-glucose incubation mixture under conditions optimal for the esterification of palmitate (*vide supra*).

The effect of unconjugated bile salts. In order to assess the role of the conjugation of bile salts, we investigated the effect of free bile salts on the small intestine. Specifically, we investigated the influence of cholate and desoxycholate on palmitate esterification, glucose transport and tissue histology. Free desoxycholate inhibited palmitate esterification (Figure 3), and this inhibition was demonstrable at a concentration as low as 5×10^{-4} M and was almost complete at 3×10^{-3} M. This inhibition was more readily demonstrated when palmitate esterification had been increased by the presence of a wetting agent (e.g., albumin, Tween "80" or taurocholate). The *inhibition* by free desoxycholate is in striking contrast to the *stimulation* by taurodesoxycholate which occurs at an optimal concentration of $5 \times$

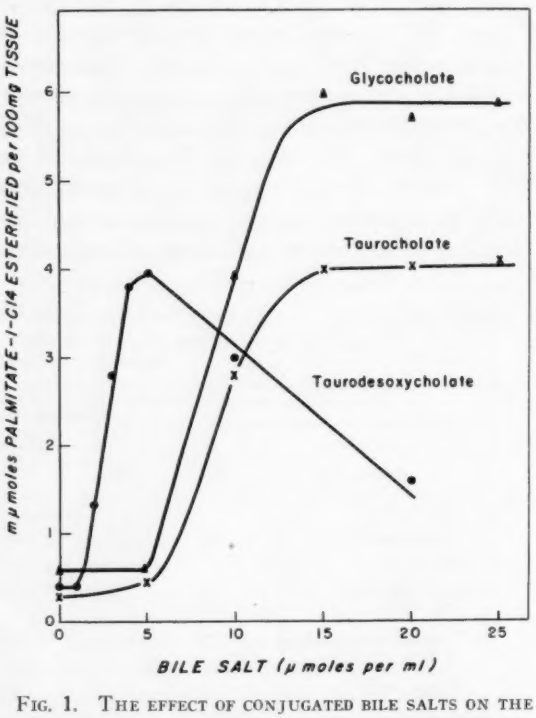


FIG. 1. THE EFFECT OF CONJUGATED BILE SALTS ON THE ESTERIFICATION OF PALMITATE-1-C^{14} BY SLICES OF RAT SMALL INTESTINE. Standard incubation procedure was used. There were 100 mμmoles (5×10^5 cpm) of palmitate-1-C^{14} in each flask and bile salts were added as indicated.

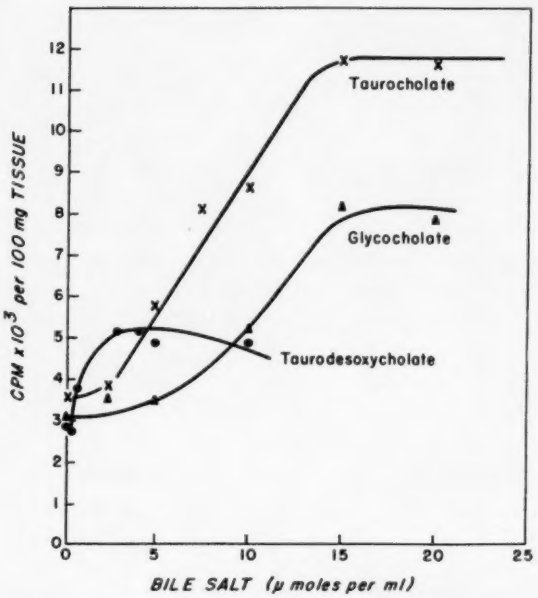


FIG. 2. THE EFFECT OF CONJUGATED BILE SALTS ON THE INCORPORATION OF RADIOACTIVITY FROM C^{14}-GLUCOSE BY SLICES OF RAT SMALL INTESTINE. Each flask contained uniformly labeled C^{14}-glucose, 0.1 μmole (2.7×10^5 cpm), and conjugated bile salts as indicated.

10^{-3} M (Figure 1). Free cholate, on the other hand, did not inhibit esterification and when its effects were compared to those of its conjugated

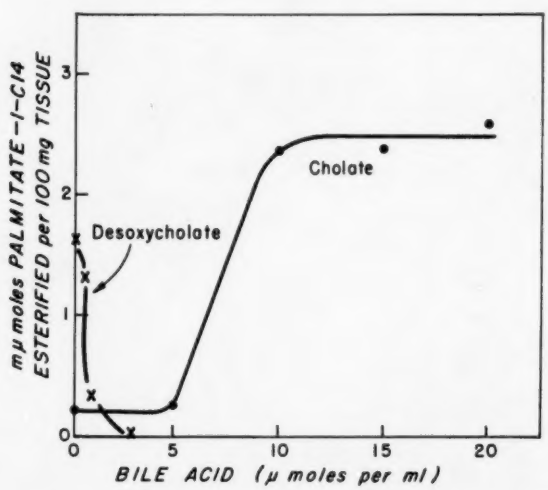


FIG. 3. THE EFFECT OF FREE BILE SALTS ON THE ESTERIFICATION OF PALMITATE-1-C^{14} BY SLICES OF RAT SMALL INTESTINE. Standard incubation conditions were used. Each flask contained palmitate-1-C^{14}, 100 mμmoles (5×10^5 cpm), and free bile salts as indicated. Each flask in the desoxycholate experiments contained Tween "80" (0.38 per cent) to facilitate esterification.

TABLE VIII

Comparison of the effect of cholate and its conjugated derivatives on the esterification of palmitate-1-C^{14} by slices of rat small intestine *

Bile salt		Maximal palmitate-1-C^{14} esterified/ 100 mg tissue
Type	Optimal concentration	
	$\times 10^{-3}$ M	*mμmoles*
Cholate	10	2.8
Glycocholate	15	3.9
Taurocholate	15	3.7

* Standard incubation procedure. Data in column 2 are derived from Figures 1 and 3. Data in column 3 are derived from the action of the bile salts on tissue from the same rat.

derivatives (Table VIII, Figures 1 and 3) there were two obvious differences: *1*) the maximal palmitate esterification produced by cholate was less than that produced by its conjugate when tested on tissue from the same animal (Table VIII, column 3); and *2*) the concentration at which this maximum was reached was lower for cholate (Table VIII, column 2). To be certain that these observations were not due to unrecognized contaminants, these results were confirmed

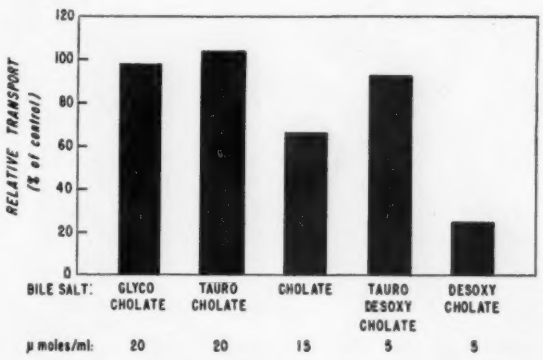
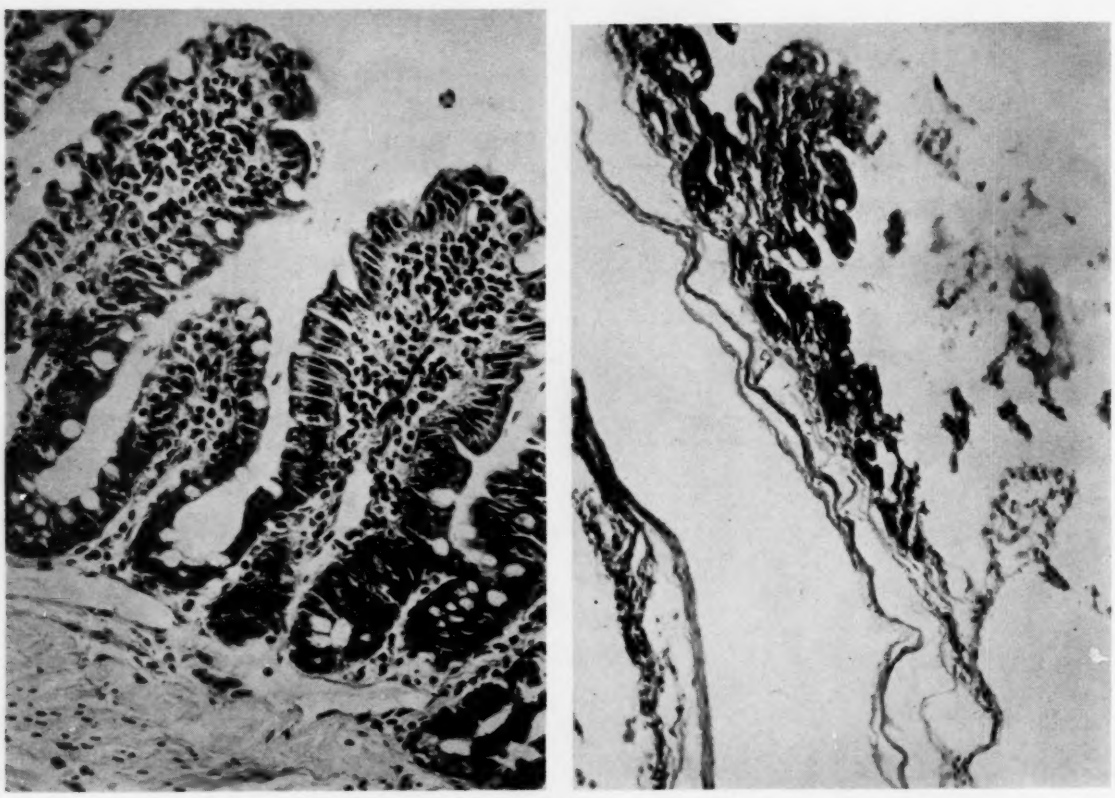


FIG. 4. THE EFFECT OF BILE SALTS ON THE TRANSPORT OF THE C^{14}-GLUCOSE BY EVERTED SACS OF RAT SMALL INTESTINE. Krebs-Ringer phosphate buffer containing unlabeled glucose (10^{-3} M) was used for the incubation medium and for the sac contents. A tracer dose of C^{14}-glucose (0.02 μmole, 2.25×10^5 cpm) was added to the incubation medium. Bile salts were added as indicated. The final volume of the incubation medium was 4 ml and the incubation was for one hour at 25° C under oxygen. At the end of the incubations, aliquots of the sac contents were dried on planchets and counted in a gas-flow counter. The control sacs without bile salts contained on an average 6.5×10^5 cpm per ml at the end of the incubation. The results of the incubation with bile salts are expressed as per cent of the controls. Each result represents the mean value of at least eight sacs.

with free acids obtained by the hydrolysis of recrystallized conjugated bile salts.

The inhibition by desoxycholate was not specific for the esterifying activity of the mucosa as shown by experiments on glucose transport using everted sacs of small intestine. It was found (Figure 4) that whereas taurodesoxycholate (5×10^{-3} M) had no effect on glucose transport, free desoxycholate at this concentration decreased glucose transport by 75 per cent. Cholate (1.5×10^{-2} M) inhibited glucose transport 35 per cent, while its conjugated derivatives, glycocholate and taurocholate (2×10^{-2} M), had no effect on this process. These results on the interference of intestinal glucose transport by free bile salts emphasize the limitation of using C^{14}-glucose to assess intracellular metabolism when the results depend upon the initial entry of the label into the cell.

Normally, at the end of a one hour incubation of rat small intestine at 25° C in buffer containing glucose (10^{-3} M), histological sections of this tissue showed generally well preserved villi with scattered mild mucosal damage. The results were similar when taurodesoxycholate (5×10^{-3} M) was in the medium (Figure 5A). On the other hand, free desoxycholate (5×10^{-3} M) caused dissolution of the tissue with loss of whole villi (Figure 5B). Even at concentrations of 10^{-3} M all villi had necrotic tips. The effect of cholate was far less pronounced, but at 1.5×10^{-2} M it caused histological damage to all villi. Intestinal segments incubated with glycocholate or taurocholate (2×10^{-2} M) were histologically the same



A B

FIG. 5. THE EFFECT OF FREE DESOXYCHOLATE AND TAURODESOXYCHOLATE IN THE HISTOLOGY OF RAT SMALL INTESTINE. The sacs of small intestine used to demonstrate glucose transport (Figure 4) were fixed in 10 per cent formalin and histologic sections prepared and stained with hematoxylin and eosin. A) After incubation with taurodesoxycholate, 5×10^{-3} M (magnification $\times 200$); B) after incubation with desoxycholate, 5×10^{-3} M (magnification $\times 100$).

TABLE IX

The separation of labeled small intestinal lipids by silicic acid chromatography *

Eluate		Lipid fraction eluted	Radioactive lipid following incubation with			
			Palmitate-1-C^{14}		C^{14}-glucose	
Solvent	Volume					
	ml†		*cpm*	%	*cpm*	%
1% Benzene	18	Cholesterol ester	42	0.2		
3% Ether in hexane	60	Triglyceride	19,846	86.8	23,913	92.0
30% Ether in hexane	60	Diglyceride	2,360	10.4	1,691	6.5
Ether	60	Monoglyceride	224	1.0	200	0.7
Methanol	100	Phospholipid	336	1.5	242	0.9

* The lipid was prepared from rat small intestine following a standard incubation with either palmitate-1-C^{14} or uniformly labled C^{14}-glucose in the presence of taurocholate 2×10^{-2} M. All extractions were performed under nitrogen and the lipid was not heated to more than 37° C. The lipid from the glucose experiment was not passed through an Amberlite IRA-400 column prior to silicic acid chromatography.

† The eluate was collected in 6 ml fractions and good separation of the respective lipid peaks was demonstrated. For the sake of brevity the total volume of each solvent fraction and the total radioactivity in each fraction are given.

as the controls. Similar experiments were also performed with everted segments of rat colon. The free bile salts again caused some mucosal damage but to a considerably lesser extent than that seen in the rat small intestine.

Identification of labeled incubation products. The results of silicic acid chromatography of the labeled lipid formed during an incubation with either palmitate-1-C^{14} or uniformly labeled C^{14}-glucose are shown in Table IX. With labeled lipid derived from palmitate-1-C^{14} experiments, 0.2 per cent of the counts were eluted with 1 per cent benzene in hexane and probably represented cholesterol ester. Over 85 per cent of the label was in the triglyceride fraction, about 10 per cent in the diglyceride fraction, and 1 to 2 per cent in both monoglyceride and phospholipid. In view of the very small proportion of counts in the lower glyceride fractions, their identity was also confirmed by chromatography on silicone impregnated paper (14). Some of the monoglyceride was found to come from hydrolysis of higher glycerides on the IRA-400 column. The monoglyceride due to such hydrolysis amounted to 0.5 per cent of the total counts in the tri- and diglyceride fractions. Nevertheless, it is evident in Table IX that in the C^{14}-glucose experiments (where IRA columns were not used) the labeled lipid still showed a definite, though small (0.7 per cent), monoglyceride fraction.

DISCUSSION

These observations on factors affecting lipid metabolism in rat small intestine have been facilitated by using a reproducible technique for measuring both palmitate-1-C^{14} esterification and the incorporation of label from C^{14}-glucose into intestinal lipid. A somewhat similar system for measuring palmitate esterification has recently been described by Johnston (15). He used everted sacs of hamster small intestine which were incubated at 37° C for 2.5 hours with a carefully prepared albumin-palmitate-1-C^{14} complex. Under his conditions, in contrast to our experiments, a significant proportion of esterified palmitate-1-C^{14} was transported into the serosal medium. This difference may be related to the fact that he used a higher temperature, incubated for a longer time, and used a different species. From our observations the species difference is probably the most important, for Table IV shows that hamster jejunum is approximately seven times as active as that of the rat.

The observation that in the absence of a wetting agent palmitate-1-C^{14} adheres to the mucosal cell surface and that this process is not energy-dependent has been observed in other cells (16, 17). Goodman (16) found that red blood cells have a strong binding affinity for palmitate-1-C^{14} and that this property was also demonstrable with red cell ghosts or cyanide-poisoned cells. The reason for the negligible transport of palmitate into the mucosal cell, despite its adherence to the cell surface, is not known but was also described by Fillerup, Migliore and Mead (17) using ascites tumor cells. They facilitated the transport of palmitate into these cells by using an albumin-palmitate complex. In our system taurocholate and Tween "80" had this effect, as well as albu-

min, which suggests that albumin acts by increasing the solubility of the fatty acid rather than by any other specific property. Johnston's observations that only 10 to 20 per cent of the palmitate-1-C^{14} present in the tissue was unesterified may be explained by the fact that he used an albumin-palmitate complex in which all the palmitate was bound to the albumin. In our experiments not all the palmitate present was bound to albumin since we added the palmitate and albumin to the incubation medium for only 15 minutes before the tissue was added.

Bile salts are known to be of importance in fat absorption (10). Their postulated role is usually confined to that of a natural emulsifier of fat in the intestinal lumen. Their detergent action may or may not also explain their ability to activate pancreatic lipase (1). However, Verzar and McDougall (18) did suggest that bile salts might be of importance on the mucosal cell surface and Borgström (19), on the basis of balance studies in rats with bile and lymph fistulae, inferred that these salts had an intracellular function. Further evidence for such an action is that 24 hours after an injection of C^{14}-labeled cholate the major portion of the label remaining in the tissues was present in the small intestinal mucosa (20).

In view of the fact that Tween "80" and albumin increase the esterification of palmitate-1-C^{14} in our system, it was not surprising that bile salts which are also active surface agents would behave in a similar manner. But this cannot be the sole explanation of their effect. The preincubation experiments demonstrate that the conjugated bile salts tested, namely, taurocholate, glycocholate and taurodesoxycholate, have the property of stimulating the cellular phase of palmitate esterification. The site of such action might be on the cell surface, inside the cell or both. Recently pinocytosis has been described as an important mode of membrane transport for widely differing substances (21–24). The electron microscopic studies of Palay and Karlin (24) have suggested that pinocytosis is involved, at least in part, in the transport of fat across the rat small intestine following the feeding of corn oil. Pinocytosis may be stimulated by various factors in different tissues; e.g., insulin has been shown to induce pinocytosis in fat cells (23). It is possible that conjugated bile salts have an analogous effect on the intestinal mucosa and so stimulate the transport of palmitate into the cell making it available for esterification. Regardless of this highly speculative cellular surface role of conjugated bile salts, the experiments with C^{14}-glucose show that these salts affect intramucosal lipid metabolism. A recent report by Whitehouse and Staple (25) provides further evidence that conjugated bile salts have an intracellular metabolic role.

It is of interest that at low concentrations these bile salts had no obvious effects. The observation that two conjugated bile salts can act in a complementary manner when present at low concentrations is of interest, for the bile of most species contains a mixture of these salts.

The difference between the biological properties of free bile salts and their conjugates has not been emphasized in the past, although pharmacological differences have been described (26). This difference was best demonstrated in our experiments by comparing the effect of taurodesoxycholate and desoxycholate on palmitate-1-C^{14} esterification, glucose transport, and tissue histology. The derangement in cell structure should perhaps not be surprising since this agent is frequently used to disrupt cell fractions *in vitro* (27). The difference between cholate and its conjugates was less impressive but nevertheless definite, especially with regard to the effect on glucose transport and cell structure. Whitehouse and Staple (25) also found that cholate and its conjugated derivatives differed in their metabolic effects in a cell-free system in that cholate inhibited pyruvate oxidation by rat liver mitochondria while its conjugated derivatives had no such effect.

Investigations on the physiology of absorption when crude bile salts are used must be interpreted with caution. Unless stringent tests of purity are employed the results may be misleading, for traces of unconjugated bile salts (especially desoxycholate) can alter mucosal function. We encountered this situation when testing both a crude ox bile preparation and a so-called "chemically pure" glycocholate preparation. Both substances contained free bile salts and both inhibited palmitate esterification. These findings might also partially explain some of the confusion concerning the therapeutic usefulness of bile salts

in correcting impaired fat absorption in patients and rats with biliary fistulae.

The conjugation of bile salts which occurs in the liver immediately after they are synthesized from cholesterol serves to convert potentially harmful substances into physiologically useful ones. Normally the small intestine contains only conjugated bile salts. Some of these are absorbed by the small intestine while others pass into the large bowel where they are rapidly hydrolyzed and degraded by bacteria (8, 9, 28).

In the clinical condition of intestinal "blind loops," it has been assumed that an altered small bowel flora contributes to the associated malabsorption (29). It is tempting to speculate that in circumstances where bacteria infest the small bowel conjugated bile salts may be converted in the lumen of the small intestine to toxic unconjugated derivatives. These products might then interfere with absorption, especially since it is known that one of the initial bacterial degradation products of cholate is desoxycholate.

In our analyses of the labeled lipid formed during an incubation, we consistently found a small proportion of the label (1 to 2 per cent) in the monoglyceride fraction. This monoglyceride fraction is of interest for it would not be expected as an intermediate in the formation of triglyceride from fatty acids if Kennedy's scheme for the conversion of fatty acids to neutral glycerides obtains in the mucosa (30). In fact the monoglyceride probably represents a product of lipolysis rather than of lipogenesis. Active mucosal lipolysis has been demonstrated in the rat *in vivo* (12) and in rat mucosal homogenates (3).

The action of the conjugated bile salts in the homogenate system (3) was different from that of the tissue slice. In the homogenate, taurocholate depressed the incorporation of palmitate-1-C^{14} into neutral fat. This was interpreted as being due to the activation of lipase. The difference between the results using these two techniques probably is due to the abnormal structural relationships of cellular particles in the homogenate system. Thus, although homogenates are of use in delineating reactions and their cofactor requirements, the use of intact cells is mandatory to evaluate the possible physiological role of such reactions.

SUMMARY

1. A reproducible method has been described for studying aspects of lipid metabolism in slices of rat small intestine *in vitro*.

2. When slices of rat small intestine are incubated with only palmitate-1-C^{14} and buffer, fatty acid predominantly adheres to the cell surface and negligible esterification occurs. This binding is independent of the metabolic activity of the cell.

3. Esterification of palmitate by slices of intestine is facilitated by adding a wetting agent such as albumin, Tween "80" or taurocholate to the medium. This esterification is dependent upon the metabolic activity of the cell.

4. Conjugated bile salts stimulate the esterification of palmitate-1-C^{14} by directly affecting mucosal cell metabolism in addition to their effect on fatty acid solubility in the incubation medium. This was demonstrated by preincubation studies and by experiments in which conjugated bile salts stimulated the incorporation of radioactivity from C^{14}-glucose into mucosal lipid.

5. The free bile salts, cholate and desoxycholate, behave differently from their conjugated derivatives. The free salts inhibit glucose transport by the small intestine and cause histological damage. Desoxycholate also inhibits palmitate esterification, but cholate stimulates this process. However, the stimulation of palmitate esterification by cholate is less than that produced by glycocholate or taurocholate.

6. Some possible physiological and pathological implications of these findings have been discussed.

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THE METABOLISM OF CORTICOSTERONE IN MAN

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Corticosterone was the first biologically active hormone to be isolated from adrenal gland extracts (1, 2). Although contributing little to the body pool of adrenocortical steroids in man, it is one of the major adrenocortical secretory products in some animals, e.g., rodents (3). In man the major glucocorticosteroid is cortisol and the major mineralocorticosteroid is probably aldosterone; nevertheless, corticosterone has been identified and measured in human adrenal tissue (4), adrenal gland perfusates (5), adrenal vein blood (6–8), and peripheral blood (9–16). In all of these tissues the corticosterone concentration was found to be lower than that of cortisol, although the concentration of the biologically important aldosterone has been found to be even less than that of the corticosterone (17, 18).

Unlike cortisol, little is known about the rates of secretion of corticosterone in the normal subject, or its alteration in disease. This paucity of knowledge stems largely from the fact that there are no readily available quantitative or semiquantitative methods for determining even a single fraction of the metabolites of corticosterone in the urine.

In previous reports we have described studies on the physiological disposition and metabolic fate of cortisol (19) and cortisone (20) in man. This paper describes similar studies with corticosterone using corticosterone, corticosterone-4-C^{14}, and H^3-labeled corticosterone.[1]

MATERIALS AND METHODS

Seventeen normal subjects, one bilaterally adrenalectomized subject, and patients with cirrhosis, myxedema, and thyrotoxicosis were studied.

Corticosterone was prepared for intravenous administration by dissolving 50, 100 or 200 mg of the crystalline steroid in 10 to 15 ml ethanol, which was added to 300 ml sterile 5 per cent dextrose in water. The solution was infused over a period of 10 to 20 minutes. The orally administered corticosterone was suspended in saline or given in a gelatin capsule. Corticosterone-4-C^{14} (0.004 μc per μg) was administered intravenously in trace quantities (100 to 400 μg) in a small volume of 5 per cent ethanol in sterile distilled water over a period of 5 minutes. Corticosterone-H^3 (1.5 μc per μg) was administered in trace quantities (7 to 30 μg) in a small volume of 5 per cent ethanol in sterile distilled water. After injection of the steroids, heparinized blood samples were collected at 20 to 30 minute intervals.

The extraction procedures for the plasma corticosterone were similar to those described previously for cortisol (19). Following the infusion of the pharmacological quantities of the steroid, two different methods were used to determine the plasma corticosterone concentration.

Fluorometric assay. Five-tenths ml plasma was extracted with 5 ml dichloromethane [purified by passage through a column of silica gel (21)]. The plasma was removed by aspiration, and the dichloromethane extract

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[1] In this paper the trivial names of the steroids have been used: cortisol (11β,17α,21-trihydroxy-4-pregnene-3,20-dione); cortisone (17α,21-dihydroxy-4-pregnene-3,11,20-trione); corticosterone (11β,21-dihydroxy-4-pregnene-3,20-dione); tetrahydrocortisone (3α,17α,21-trihydroxy-pregnane-11,20-dione); tetrahydrocortisol (3α,11β,17α,21-tetrahydroxy-pregnane,20-one); tetrahydrocorticosterone (3α,11β,21-trihydroxy-pregnane-20-one); 9α-fluorocortisol (9α-fluoro-11β,17α,21-trihydroxy-4-pregnene-3,20-dione); Δ^1-9α-fluorocortisol (9α-fluoro-11β,17α,21-trihydroxy-1,4-pregnene-3,20-dione); 11-dehydrotetrahydrocorticosterone (3α,21-dihydroxy-pregnane-11,20-dione); 3α-allotetrahydrocorticosterone (3α,11β,21-trihydroxy-allopregnane-20-one); 11-dehydroallotetrahydrocorticosterone (3α,21-dihydroxy-allopregnane-11,20-dione); 11-dehydro-corticosterone (21-hydroxy-4-pregnene-3-11,20-trione); 11-desoxycortisol (17α,21-dihydroxy-4-pregnene-3,20-dione); 20α-hydroxycorticosterone (11β,20α,21-trihydroxy-4-pregnene-3-one); 20β-hydroxycorticosterone (11β,20β,21-trihydroxy-4-pregnene-3-one); 11-epicorticosterone (11α,21-dihydroxy-4-pregnene-3,20-dione); allotetrahydrocortisol (3α,11β,17α,21-tetrahydroxy-allopregnane-20-one); allotetrahydrocortisone (3α,17α,21-trihydroxy-allopregnane-11, 20-dione); Δ^1-9α-fluoro-16α-hydroxycortisol diac (16α,21-diacetoxy-11α,17α-dihydroxy-9α-fluoro-1,4-pregnene-3,20-dione); 2α-methylcortisone (2α-methyl-11β,17α,21-trihydroxy-4-pregnene-3,20-dione); 20β-hydroxycortisone (17α,20β,21-trihydroxy-4-pregnene-3,11-dione).

washed with 0.5 ml 0.1 N NaOH. Each of two 2 ml aliquots of the extract was transferred to a small conical test tube and shaken with 1 ml of a sulfuric acid-ethanol (redistilled ethanol) reagent (65:35 vol/vol). The solvent was removed by aspiration and the acid-alcohol reagent transferred to a small cuvet for fluorescence determination in a Farrand fluorometer by a previously described modification (12) of the method of Sweat (22).

Isotope dilution assay. The plasma samples were also assayed for corticosterone by the isotope dilution procedure as previously described (12), except that 5 ml of plasma was used for each determination. Radioactivity measurements were made in a Tri-Carb liquid scintillation spectrometer (Packard Instrument Co.).

Determination of plasma corticosterone-H^3 and labeled metabolites. The disappearance of H^3-labeled corticosterone from the plasma was determined by first adding a known number of counts of corticosterone-4-C^{14} (500 cpm at an efficiency of 30 per cent) to a known volume of each plasma sample collected after the infusion of the labeled steroid. The plasma was extracted with dichloromethane and the residue chromatographed in a modified Bush-type system (23) (cyclohexane 100: benzene 100: methanol 100: water 50) for 24 hours at room temperature using unlabeled corticosterone (20 μg) as a marker. The corticosterone was then located with ultraviolet light, eluted, and assayed for C^{14} and H^3 in a liquid scintillation spectrometer. The C^{14} in this case was used to correct for losses of corticosterone-H^3 during the extraction, evaporation, chromatography, and elution steps. The mixtures of C^{14} and H^3 were counted by previously described methods (24). The combined plasma "free" corticosterone and its metabolites were measured by direct counts of an aliquot of the dichloromethane extract of the plasma. The plasma radioactive metabolites of corticosterone conjugated with glucuronic acid were determined by a previously described method (19).

Determination of specific activity of labeled plasma corticosterone. Fifteen to 20 ml of heparinized plasma collected serially after injection of corticosterone-H^3 was extracted with 7 volumes of dichloromethane. The plasma was removed by aspiration, and the dichloromethane washed successively with 1/10 volume 0.1 N NaOH and 1/10 volume of 0.1 M acetic acid. The dichloromethane extract was evaporated to dryness. Twenty μg of 11-desoxycortisol was added to the residue, and this was applied to Whatman no. 1 filter paper and chromatographed (cyclohexane 100: benzene 100: methanol 100: water 50) for 24 hours. After the chromatography the strip was scanned with ultraviolet light, and the 11-desoxycortisol located. (Corticosterone in 24 hours runs 2 to 3 cm slower than 11-desoxycortisol in this system.) The 3 to 4 cm portion of the paper just proximal to 11-desoxycortisol was cut out and the corticosterone eluted with ethanol. The alcohol was evaporated to dryness in a small, conical, glass-stoppered test tube.

The dry residue was acetylated with equal parts of 15 per cent (vol/vol) acetic anhydride-1-C^{14} (2 mc per mmole) in benzene and in pyridine for 24 hours at room temperature. The unused acetic anhydride was removed by partitioning twice between a small volume of 20 per cent ethanol and dichloromethane. The aqueous layer was removed by aspiration into an alkali trap. After addition of 20 μg of corticosterone acetate, the dichloromethane was evaporated to dryness. The residue was chromatographed successively in the following systems: *1*) cyclohexane 100: benzene 50: methanol 100: water 25, 15 hours; *2*) cyclohexane 100: dioxane 100: methanol 50: water 25, 18 hours; and *3*) cyclohexane 100: benzene 50: methanol 100: water 25, 15 hours. After elution from the second chromatographic purification and prior to the third and final chromatography, the residue was oxidized to 11-dehydrocorticosterone acetate with 0.1 ml 0.5 per cent CrO_3 in redistilled glacial acetic acid. After exposure to the CrO_3 for 1 minute at room temperature, 1 ml of 20 per cent ethanol-water was added and the steroid acetate was extracted with dichloromethane. The dichloromethane was evaporated to dryness and the residue applied to paper for the third chromatographic purification.

The 11-dehydrocorticosterone acetate was eluted into a counting vial, dried, and 5 ml toluene containing 0.4 per cent DPO (diphenyloxazole) and 0.004 per cent POPOP [1,4-*bis*-2-(5-phenyloxazolyl)-benzene] phosphor added. The samples were assayed for C^{14} and H^3 in a liquid scintillation spectrometer. The calculations of the specific activities have been described previously (24). From a knowledge of the specific activity of the acetic anhydride-1-C^{14} and the C^{14} counts in the counting vial, it was possible to determine the micrograms of corticosterone in the vial. From the counts of tritium and the micrograms of corticosterone in the vial, the specific activity of the corticosterone was determined. The fractional rate of turnover of corticosterone and the miscible pool size were determined as previously described for cortisol (25).

Comparison of in vivo metabolism of H^3- and C^{14}-labeled corticosterone. A solution of 320 μg corticosterone labeled with both H^3 and C^{14} was injected into a normal subject (N. K.) who was receiving a continuous intravenous infusion of corticotropin (4 units per hour) for 36 hours. The labeled steroid was given 12 hours after the start of the infusion. Blood samples were collected every 30 minutes for 3 hours. The plasma corticosterone specific activities were determined as previously described (isotope dilution assay utilizing measurements of fluorescence), except that both radioactive carbon and tritium measurements were made. The ratio of specific activities of the carbon and tritium were constant for the 3 hours of the study, thus indicating that the C^{14}- and H^3-labeled corticosterone were probably handled in a similar manner (Figure 1).

The C^{14}- and H^3-labeled urinary metabolites were also assayed in this same subject, and no significant differences were found in the rate of excretion of the administered C^{14} and H^3 in the total, free, and glucuronide-con-

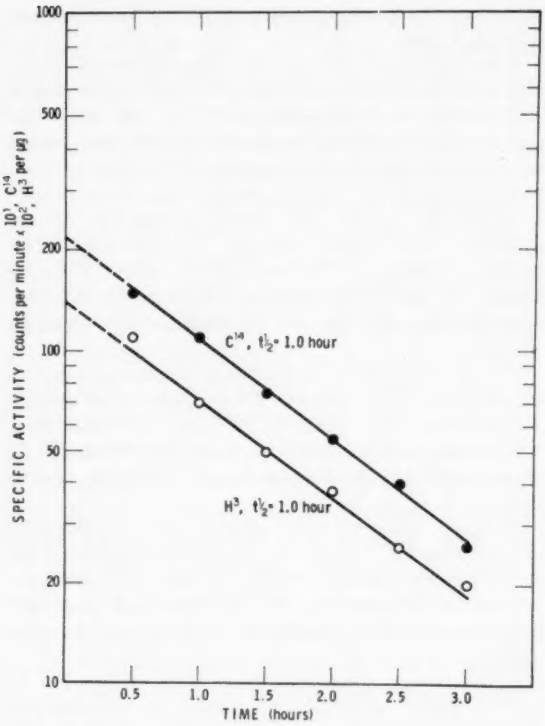

FIG. 1. SPECIFIC ACTIVITY OF CORTICOSTERONE IN PLASMA FOLLOWING INTRAVENOUS ADMINISTRATION OF 320 µG OF CORTICOSTERONE LABELED WITH H^3 AND C^{14}, GIVEN DURING INFUSION OF CORTICOTROPIN.

jugated metabolites (18). Studies of the plasma fractions (total, free, and glucuronide) following the infusion of a mixture of H^3- and C^{14}-labeled corticosterone also showed no differences in the H^3 to C^{14} ratios (18). Determinations were made of the C^{14} and H^3 in the corticosterone, tetrahydrocorticosterone, allotetrahydrocorticosterone, and 11-dehydrotetrahydrocorticosterone isolated from the urine. The labeled corticosterone and 11-dehydrotetrahydrocorticosterone were diluted with their respective carrier steroids and recrystallized twice, and the C^{14} and H^3 content again determined. It was not possible within the experimental error of the methods for counting the mixtures of H^3 and C^{14} to observe any significant differences in the ratios of tritium to carbon in the urinary corticosterone or the metabolites of corticosterone. The ratio of H^3 to C^{14} in all cases approximated the H^3 to C^{14} ratio in the administered corticosterone.

Daily rate of turnover of corticosterone by assay of specific activity of urinary metabolites. The specific activities of urinary corticosterone, tetrahydro- and allotetrahydrocorticosterone, and 11-dehydrocorticosterone were determined on the 24 hour collections of urine after the infusion of trace quantities of H^3-labeled corticosterone (7 to 30 µg), or slightly larger than trace quantities of corticosterone-4-C^{14} (100 to 400 µg). The labeled corticosterone and its metabolites were presumed to be diluted only by the corresponding unlabeled steroid and its metabolites, and the collection of radioactivity in the metabolites was presumed to be nearly complete within the first 24 hours. From a knowledge of the specific activity of the isolated steroid and the quantity and specific activity of the injected steroid, plus a record of the time of the urine collection after administration of the labeled steroid, it was possible to calculate the rate of steroid secretion (17, 18, 26, 27):

$$\left(\frac{I_i(f)}{I_0} - 1\right) a \times \text{no. days} = \text{micrograms of steroid secreted per day,}$$

where I_i = specific activity of injected steroid; f = ratio molecular weight of injected steroid to molecular weight of steroid metabolite; I_0 = specific activity of diluted urinary steroid metabolite; a = micrograms of steroid injected.

The specific activities of urinary steroid metabolites were determined by the following method: one hundred to 200 ml of urine was treated with Sigma bacterial β-glucuronidase (50 units per ml) for 24 hours at 37° C, and the urine was extracted with 7 volumes of dichloromethane. The dichloromethane was washed with alkali and acetic acid and evaporated to dryness as previously described. The residue was dissolved in a small volume of ethanol, and water added to a concentration of 25 per cent ethanol-water. This was extracted with 5 volumes of petroleum ether. The ether was removed by aspiration, and the aqueous solution extracted with 7 volumes of dichloromethane. The aqueous layer was removed by aspiration and the dichloromethane evaporated to dryness.

The extract was chromatographed in the cyclohexane 100: benzene 100: methanol 100: water 50 system for 20 hours. The tetrahydro metabolites were located by use of a strip counter for detection of radioactivity, or by the aid of added steroid markers visible in ultraviolet light, with running rates close to or identical with the metabolite of interest: corticosterone 27 cm; 3α-tetrahydrocorticosterone, 16 cm; Δ^1-9α-fluoro-16α-hydrocortisol diac, 16 cm; 3α-allotetrahydrocorticosterone, 20 cm; 2α-methyl-cortisone, 21 cm; 3α,11-dehydrotetrahydrocorticosterone, 25 cm; corticosterone, 27 cm; 3α,11-dehydroallotetrahydrocorticosterone, 31 cm; 11-desoxycortisol, 30 cm.

After the steroid metabolites were eluted separately, they were acetylated with labeled acetic anhydride [H^3-labeled acetic anhydride (100 mc per mmole) was used for the C^{14}-labeled metabolites, and acetic anhydride-1-C^{14} (5 mc per mmole) for the H^3-labeled metabolites]. The labeled acetylated derivatives were purified by paper chromatography in three different systems to remove all traces of contaminating radioactivity: *1*) cyclohexane 100: benzene 25: methanol 100: water 10, 12 hours; *2*) cyclohexane 100: nitromethane 50: methanol 50, 48 hours; and *3*) cyclohexane 100: methanol 100: water 10, 20 hours.

Prior to the final chromatography, all of the derivatives were converted to the 11-oxo compounds by mild oxidation with CrO_3. The diacetates of the 3α,5α (allo) compounds had a slower mobility than the 3α,5β isomers.[2]

The final purified steroid acetates were assayed for tritium and carbon radioactivity in a liquid scintillation spectrometer. From a knowledge of the specific activity of the labeled acetic anhydride plus the radioactive counts contributed to the final counting sample by the labeled acetoxy groups, it was possible to calculate the quantity of steroid in the counting vial. From the radioactive counts of the second (injected) steroid, plus the micrograms of steroid counted, it was possible to determine the specific activity of the urinary steroid metabolite after dilution by the endogenous steroid. As with all isotope dilution assays, the results were not invalidated by failure to obtain quantitative recoveries through any of the steps of the procedure.

Quantitative determination of urinary metabolites of corticosterone and cortisol. A known aliquot of the 24 hour urine was treated with Sigma bacterial β-glucuronidase (100 units per ml urine, pH 6.2) for 24 hours at 37° C. The H^3-labeled steroid tetrahydro metabolites[3] were added, and the urine extracted with dichloromethane. This extract was washed with alkali and acetic acid and evaporated to dryness. The residue was partially purified as previously described above. The residue used for the corticosterone metabolites was chromatographed in the cyclohexane 100: benzene 100: methanol 100: water 50 system for 24 hours. The free steroid metabolites were located as described above. The extracts for the cortisol metabolites were chromatographed in the benzene 100: methanol 50: water 25 system for 24 hours. The following steroid markers were used: cortisol, 32 cm; 3α-tetrahydrocortisol, 16 cm; Δ^1-9α-fluorocortisol, 15 cm; 3α-allotetrahydrocortisol, 18 cm; 20β-hydroxycortisone, 20 cm; 3α-tetrahydrocortisone, 27 cm; 9α-fluorocortisol, 26 cm; 3α-allotetrahydrocortisone, 31 cm; cortisol, 32 cm.

After chromatography the free compounds were acetylated with labeled acetic anhydride-1-C^{14} (5 mc per mmole for corticosterone metabolites and 1.0 mc per mmole for cortisol metabolites) and the derivatives chromatographed in three different solvent systems. The corticosterone metabolites were chromatographed in the three systems described above, and the cortisol metabolites in the following three solvent systems: *1*) cyclohexane 100: benzene 25: methanol 100: water 10, 24 hours; *2*) cyclohexane 100: dioxane 25: methanol 100: water 25, 48 hours; *3*) cyclohexane 100: benzene 25: methanol 100: water 10, 24 hours.

The monoacetate of cortisol was chromatographed in the following three systems: *1*) cyclohexane 50: benzene 100: methanol 100: water 20, 12 hours; *2*) cyclohexane 100: dioxane 100: methanol 50: water 25, 30 hours; *3*) carbon tetrachloride 100: methanol 100: water 25, 15 hours.

With all steroids CrO_3 oxidation preceded the third chromatography. After the final chromatography, the eluted steroids were assayed for C^{14} and H^3 in the liquid scintillation spectrometer, and the concentrations of urinary steroids were determined as previously described (24).

Determination of labeled urinary metabolites. The labeled urinary and biliary metabolites of corticosterone-4-C^{14} were measured by previously described methods (19, 20), except that with the corticosterone-H^3 and some of the corticosterone-4-C^{14} studies the radioactive measurements were made with a liquid scintillation spectrometer. A small aliquot of urine (0.1 to 0.5 ml) was added directly to 10 ml toluene, phosphor, and enough ethanol to make the mixture miscible (2 to 6 ml). A correction for loss of efficiency due to "quenching" was made by utilizing a double isotope differential dilution technique (30).

RESULTS

Rate of disappearance of corticosterone and "corticosterone-like" material from the plasma after intravenous administration. When the serial concentrations of sulfuric acid-induced fluorescent material in the dichloromethane extracts of plasma after the infusion of corticosterone were plotted against time on semilogarithmic paper, a linear decline in plasma concentration was noted (Figure 2). In some of the subjects this first order decline in plasma concentration did not begin until 30 to 60 minutes after the end of the infusion. The decline in plasma concentration during the 30 to 60 minutes after the end of the infusion was rapid and did not represent a single exponential. When corticosterone was assayed by the more selective isotope dilution method, the nonlinear

[2] A more complete description of the chromatography systems will be described in a forthcoming publication. The monoacetate of corticosterone was chromatographed in the three solvent systems described above for determination of specific activity of labeled plasma corticosterone.

[3] H^3-labeled cortisol (1.0 μc per μg) and corticosterone (1.5 μc per μg) were prepared by exposure of 1.5 mg of the dry steroid acetates to H^3 gas. The irradiated steroids were purified by paper chromatography (before and after hydrolysis of the acetates) to a constant specific activity. The 3α,5β tetrahydro H^3 compounds were prepared enzymatically from the parent steroids with the soluble dehydrogenase enzymes from rat liver (28), and the 3α,5α tetrahydro compounds were prepared from the parent H^3 steroids with the particulate enzymes from the livers of thyrotoxic rats (29). Twenty-five to 50 ml of normal urine was sufficient for the cortisol metabolites, whereas 100 to 200 ml was required for the assay of the corticosterone metabolites. Corticosterone-4-C^{14} (1.47 mc per mmole) was obtained from the Endocrine Study Section of the National Institutes of Health.

nature of the initial concentration curve was also observed. With both the sulfuric acid-induced fluorescence and the isotope dilution assay the slopes of the slower linear curve of declining steroid concentrations were similar. However, in all instances the plasma levels of corticosterone as measured with the more specific isotope dilution assay were always 10 to 20 per cent lower than the levels obtained with the less selective fluorometric assay.

In one normal subject 0.4, 50, 100 and 200 mg corticosterone were infused on separate occasions (Figure 3). The slopes of the slower curve of declining steroid concentrations were similar at the three larger dosage levels. The similar rates for metabolism of these three different concentrations of corticosterone and the first order nature of the decline in plasma concentration indicated that the rate of metabolism of infused corticosterone was proportional to concentration. Also, corticosterone and its sulfuric acid-induced fluorescent metabolites were metabolized at a similar rate. These rates of metabolism were conveniently expressed as biological half-times. Figure

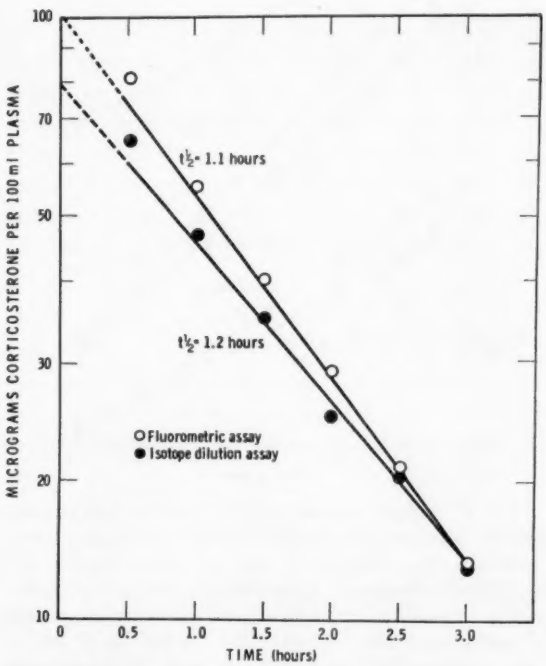

FIG. 2. DISAPPEARANCE OF CORTICOSTERONE FROM PLASMA FOLLOWING INTRAVENOUS ADMINISTRATION OF 100 MG OF CORTICOSTERONE.

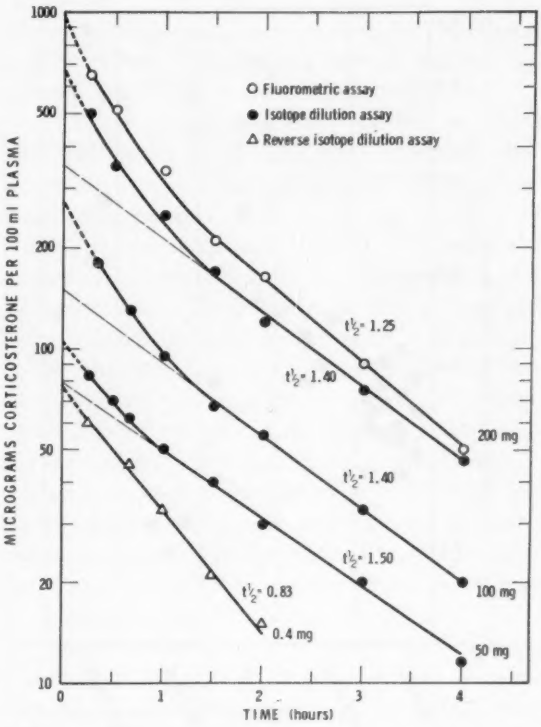

FIG. 3. DISAPPEARANCE OF CORTICOSTERONE FROM PLASMA FOLLOWING INTRAVENOUS ADMINISTRATION. THE 0.4 MG DOSE OF STEROID WAS GIVEN AS CORTICOSTERONE-4-C^{14}.

4 shows the comparative rates of metabolism of corticosterone in normal subjects, in patients with cirrhosis, myxedema, and thyrotoxicosis, and in normal subjects treated with estrogens.

In the normal subjects the biological half-times ranged from 0.9 to 1.6 hours with a mean of 1.27 hours ± 0.3 SD. A more rapid rate of metabolism was observed in three patients with thyrotoxicosis. The patients with cirrhosis and myxedema showed only a slightly delayed capacity for metabolism of infused corticosterone. Normal subjects given estrogens also showed a delayed metabolism.

Disappearance of radioactive corticosterone from the plasma. Intravenously infused tracer quantities of H^3-labeled corticosterone disappeared at a constant first order rate (Figures 3 and 5). However, the rates of disappearance of the trace and the pharmacological quantities were different. The larger amounts were metabolized at a slower rate. This would not appear to be attributable to a difference in the rate of metabolism of the H^3- and C^{14}-labeled, and unlabeled steroid. In Figure

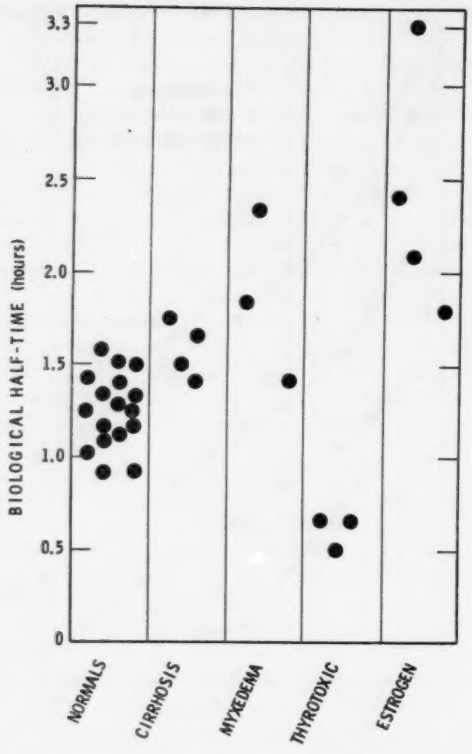

FIG. 4. BIOLOGICAL HALF-TIMES OF INTRAVENOUSLY ADMINISTERED CORTICOSTERONE (100 MG). The biological half-times were determined by fluorometric assay of the plasma samples.

5 the plasma levels, in both studies on the same subject, represent a measure of the H^3 label on the corticosterone. With the pharmacological dose, the plasma steroid concentrations were also measured by the fluorometric method and a half-time of 1.2 hours was found. The more rapid appearance in the plasma of the metabolites of corticosterone conjugated with glucuronic acid indicated that the trace dose disappeared from the plasma (metabolism) at a more rapid rate. As the corticosterone disappeared from the plasma, conjugated derivatives hydrolyzed with β-glucuronidase appeared and reached a maximum at 30 to 60 minutes, at which time the free and conjugated fractions were of similar magnitude. Thereafter the conjugated fraction exceeded the free fraction. A large portion of the free and labeled steroids in the plasma following the injection of labeled corticosterone was not corticosterone. The labeled metabolites of corticosterone disappeared at a more rapid rate than corticosterone.

Excretion of corticosterone and its metabolites. Figure 6 shows the cumulative urinary and biliary excretion of labeled corticosterone following the intravenous administration of a tracer quantity of corticosterone-4-C^{14} to a patient with a biliary cannula. The feces collected for three days following the injection of the labeled steroid did not contain any C^{14}. The total quantity of metabolites appearing in the bile over a period of 24 hours was 11 per cent. Direct dichloromethane extraction of the bile recovered only a trace of radioactivity, and thus none of the biliary metabolites represented unaltered corticosterone.

Sixty-six per cent of the injected steroid was excreted in the urine in the first 24 hours and 80

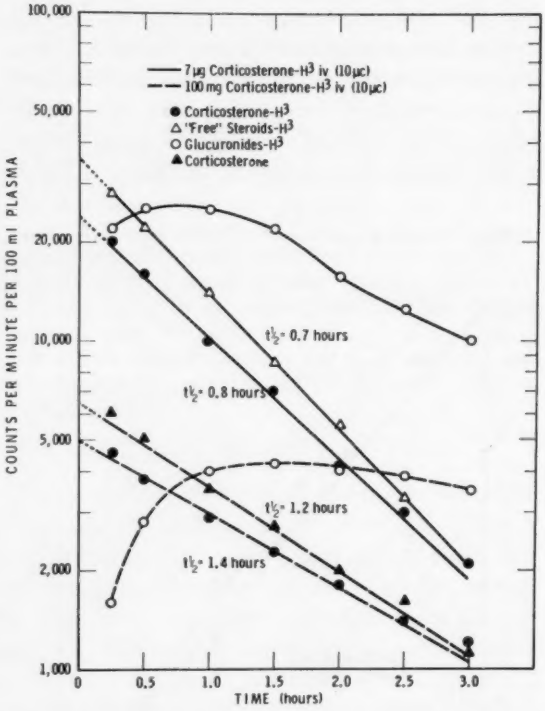

FIG. 5. CORTICOSTERONE AND GLUCURONIDE-CONJUGATED STEROID METABOLITES OF CORTICOSTERONE IN PLASMA AFTER INTRAVENOUS ADMINISTRATION OF CORTICOSTERONE-H^3. △ "Free" steroids-H^3 represents the fraction of radioactive metabolites extractable with dichloromethane and ○ glucuronides-H^3 represents the fraction of H^3-labeled radioactive metabolites extractable with dichloromethane after prior incubation of plasma with β-glucuronidase. ▲ Corticosterone was measured by direct fluorometric assay of the dichloromethane extract of the plasma.

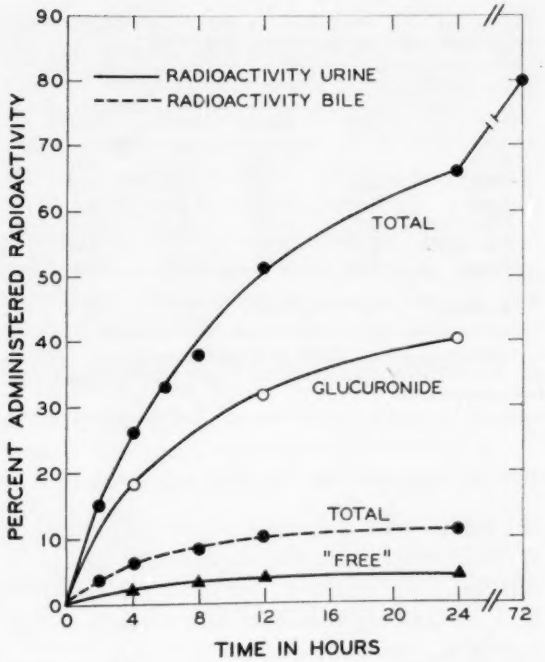

FIG. 6. CUMULATIVE URINARY AND BILIARY EXCRETION OF RADIOACTIVITY FOLLOWING INFUSION OF 3 μC OF CORTICOSTERONE-4-C^{14}.

per cent in 72 hours. By the fourth day no labeled urinary products could be demonstrated. Forty per cent of the infused steroid could be extracted from the first 24 hour urine collection after hydrolysis with β-glucuronidase, whereas only 3.5 per cent was extracted directly with dichloromethane. Incubation of the urine at pH 1.0 at 24° C for 24 hours did not make any of the labeled steroids extractable with dichloromethane. Nine per cent of the infused steroid was not accounted for in either the urine or the bile.

On other studies in normal subjects in which only the urinary metabolites were measured similar results were obtained (Table I). Most of the radioactive metabolites appeared in the first 24 hours. However, no more than 90 per cent of the administered steroid could be recovered in the 72 hour urine. Three studies in which fractional urine collections were made showed that one-half of the radioactivity excreted during the first day appeared in the urine in 2.5 to 3.2 hours. From 2 to 5.5 per cent of the labeled urinary steroid metabolites were extracted with dichloromethane without prior hydrolysis, whereas following β-glucuronidase hydrolysis and dichloromethane extraction of the urine, 40 to 60 per cent of the excreted radioactive material was recovered.

Urinary metabolites of corticosterone. The free steroid metabolites of corticosterone made up a very small fraction of the total urinary metabolites, and corticosterone accounted for only a trace (less than 0.5 per cent) of the total quantity of steroid injected. β-Glucuronidase hydrolysis of the urine released appreciable quantities of the tetrahydro metabolites of corticosterone. Table II shows the quantities of corticosterone and the tetrahydro and allotetrahydro metabolites of corticosterone in the 24 hour urine of a normal subject, and in the same normal subject given corticotropin, and 100 mg corticosterone. The data on the tetrahydro metabolites of cortisol are shown

TABLE I

Urinary excretion of labeled radiometabolites following administration of corticosterone-4-C^{14} or corticosterone-H^3

Subject	Dose	% 24 hrs	% 72 hrs	$T_{\frac{1}{2}}$	% Total excreted 24 hrs	
					Free	Glucuronide
T.R. i.v.	200 μg	70	92	2.6	5.5	60
T.R. i.v.	100 mg	60	72		3.0	53
T.R. i.v.	200 μg	70	84	3.6	3.1	45
T.R. p.o.	100 mg	58	68		3.0	45
J.B. i.v.	200 μg	66	80	2.9	3.0	60
J.B. p.o.	200 μg*	70			3.5	50
J.B. p.o.	100 mg*	30	45		2.0	40
M.P. i.v.	200 μg H^3	81	90	3.2	2.0	55
N.K. i.v.	320 μg†	70 (70)		3.0 (3.0)	2.5 (3.0)	48 (50)

* Administered as the acetate.

† Homogenous solution of a mixture of H^3 and C^{14} labeled corticosterone. Figures in parentheses represent fraction of urine metabolites labeled with H^3. This subject received a continuous infusion of corticotropin (4 units/hr) throughout the period of study.

TABLE II

Urinary excretion of metabolites of corticosterone and cortisol and rates of secretion (mg/day) *

	T.R.					N.K.			
	D.B.	Control	ACTH†	F‡		D.B.	Control	ACTH	B‡
F	0.04	0.044	2.0	1.5	B	0.005	0.002	0.31	1.0
THE	3.30	1.85	10	10	THA	0.06	0.16	1.0	3.0
THF	2.00	1.30	22	10	THB	0.12	0.27	4.0	6.1
Allo-THE	0.08	0.03	0.5	0.5	Allo-THA	0.02	0.01	0.1	0.5
Allo-THF	0.80	0.50	12	5.2	Allo-THB	0.30	0.30	5.2	9.0
Total	6.20	3.68	46.5	24.2	Total	0.50	0.74	10.6	19.6
P-S§	7.0	4.4	46	22					
Turnover	15.6	12.5	154	102	Turnover	1.3	2.3	41	99

* THE = tetrahydrocortisone, THA = tetrahydro-11-dehydrocorticosterone, THF = tetrahydrocortisol, THB = tetrahydrocorticosterone, Allo-THE = allotetrahydrocortisone, Allo-THA = allotetrahydro-11-dehydrocorticosterone, Allo-THF = allotetrahydrocortisol, Allo-THB = allotetrahydrocorticosterone.

† ACTH denotes continuous intravenous infusion of corticotropin (4 units/hr) for 36 hours. The assays were made on the urine collected from the twelfth to the thirty-sixth hour.

‡ Cortisol or corticosterone, 100 mg i.v.

§ P-S denotes urine corticoid assay (phenylhydrazine reacting chromogens after glucuronidase hydrolysis).

for comparison. Also, in these same subjects the rates of turnover of cortisol and corticosterone are listed.

Rate of turnover of corticosterone. After the injection of corticosterone-H^3, serial determination of specific activity (counts per minute per microgram) of plasma corticosterone demonstrated a rapid initial fall in specific activity. Except for a brief period (0 to 20 minutes) following injection of the tracer, essentially linear relationships were secured when the isotope content of the plasma corticosterone was plotted against time on semilogarithmic coordinates. The decline in isotopic concentration at any time was proportional to the isotopic concentration at that moment (Figure 1). From the intercepts of the plots, the specific activity of the corticosterone at the moment of mixing at time zero after injection was calculated. From this value and a knowledge of the quantity (7 to 30 μg) and specific activity (1.5 μc per μg) of the injected tracer, the magnitude of the miscible pool was calculated. In normal subjects the mean value for the miscible pool was 0.3 mg with a range of 0.20 to 0.40 mg (Table III). In normal subjects studied during the 8 to 12 hours of a continuous 48 hour intravenous infusion of corticotropin the pool size ranged from 1.5 to 3.5 mg.

From the curve of declining isotopic concentration $T_{1/2}$ was determined, and the slope of the curve, k, was calculated from the equation, $k = \log 2/T_{1/2}$. The quantity of corticosterone turned over per hour was calculated from the product of k and the pool size, and expressed as milligrams of corticosterone turned over per hour. The mean corticosterone turnover rate was 0.7 pool per hour, or 0.18 mg per hour, with a range of 0.14 to 0.34 mg per hour.

In the normal subjects given corticotropin the fractional turnover per hour was much the same as that in the normal subjects not given corticotropin; however, the pool size was increased nearly tenfold so that the rate of turnover of corticosterone was markedly increased. In the one subject in which a turnover rate study was carried out with a mixture of H^3- and C^{14}-labeled corticosterone, the results with each isotope were comparable. Serial determination of plasma corticosterone specific activities after injection of 200 μg of corticosterone-4-C^{14} (0.004 μc per μg) showed a less steep slope than that with the trace quantities of H^3-labeled corticosterone. Since the rapidly exchangeable pool of corticosterone was demonstrated to be only 0.2 to 0.4 mg, it was apparent that this amount of injected corticosterone-4-C^{14} equaled the pool size. Attempts to inject less than 200 μg met with failure because plasma radioactivity was insufficient for assay.

When the fraction of corticosterone turned over per hour was extrapolated to daily turnover by multiplying by 24, the daily rates of turnover were calculated to be more than twice the values calculated from the turnover rate method utilizing the specific activity of one or more of the urinary metabolites of corticosterone, except for the subjects receiving corticotropin. With the latter

method the daily rate of turnover was 1.5 to 4.0 mg with a mean of 2.3 mg (Table III). There were no marked differences in the results using H^3- and C^{14}-labeled corticosterone. However, with the latter, larger amounts of steroid (200 to 300 μg) were injected. In the few instances in which allotetrahydrocorticosterone or 11-dehydrotetrahydrocorticosterone were isolated in addition to tetrahydrocorticosterone, there were no significant differences in the isotopic content. In some of these same subjects corticosterone was determined and its specific activity found to be 30 to 50 per cent higher than that found in the tetrahydro compounds. In the subjects receiving corticotropin, the calculated daily turnover rates by both methods were approximately the same.

As a means of evaluating the method utilizing specific activity measurements of urinary metabolites of the labeled steroid and also the stability of the H^3 label, studies were carried out on a bilaterally adrenalectomized subject maintained on Δ^1-9α-fluorocortisol for the duration of the studies. The corticosterone-H^3 isolated from the urine collected only during the eight hours following infusion had essentially the same specific activity as the injected steroid. Thirty μg of corticosterone-H^3 plus 100 mg unlabeled corticosterone were infused into this same patient. In this study three of the tetrahydro metabolites were isolated from the 24 hour urine collection, and the calculated dilution of the administered labeled steroid closely approximated the expected value.

Absorption of corticosterone. Following the oral administration of corticosterone as the free alcohol, peak plasma corticosterone levels of 25 to 50 μg per 100 ml were usually reached at one hour, and after six hours the plasma level had returned to the baseline control value. Figure 7 shows the results of one such study in a normal fasted subject. Data are also shown for the

TABLE III

Miscible pool and rate of turnover of corticosterone *

Subject†	Age	Plasma level		Pool	$T_{\frac{1}{2}}$‡	k§		Turnover rate	
		μg %		*mg*	*hrs*		*mg/hr*		*mg/day*‖
T.R. ♂	72	0.8		0.30	1.0	0.70	0.21	THA	2.3
								THB	2.3
E.L. ♀	21	0.8		0.20	1.0	0.70	0.14		1.5
D.S. ♀	23	0.8		0.20	0.8	0.85	0.17		2.0
M.P. ♀	26	1.1		0.40	1.4	0.50	0.09		1.8
J.B. ♂	23	1.0		0.40	0.8	0.85	0.34		4.0
D.B. ♂	27	1.5		0.35	1.2	0.58	0.20		1.3
								THB	1.5
N.K. ♂	21	1.2		0.27	1.15	0.60	0.16	Allo-THB	1.5
J.E. ♂	25	0.9							3.5
L.B. ♂	21								2.6
W.R. ♂	23	1.8							3.1
L.C. ♂	23								1.8
B.S. ♀	39	0		Post-bilateral total adrenalectomy					0.1
ACTH¶									
								THA	39
N.K. 30 μg H^3		10.0		3.4	1.0	0.69	2.35	THB	43
								THA	40
D.S. 30 μg H^3		7.1		1.5	0.6	1.12	1.68	THB	40
J.B. 400 μg C^{14}		15.0		4.0	0.8	0.87	3.50		60
M.P. 400 μg C^{14}		6.0		1.2	1.2	0.58	0.70		15
			H^3	3.2	1.0	0.67	2.15		
N.K. 320 μg** $H^3 + C^{14}$		7.5	C^{14}	3.0	1.0	0.69	2.07		

* For abbreviations, see the first footnote to Table II.

† Subjects J.E., L.B., W.R., L.C. and B.S. received 200 to 300 μg of corticosterone-4-C^{14} intravenously.

‡ $T_{\frac{1}{2}}$ = time for specific activity of corticosterone to be reduced to one-half.

§ k = Fraction of pool replaced per hour.

‖ Based on specific activity of urinary tetrahydrocorticosterone, allotetrahydrocorticosterone, or 11-dehydrotetrahydrocorticosterone.

¶ ACTH = continuous intravenous infusion of corticotropin (4 units/hr) for 36 hours; labeled corticosterone injected at twelfth hour.

** A homogeneous solution of a mixture of C^{14}- and H^3-labeled corticosterone was injected at twelfth hour of infusion of corticotropin.

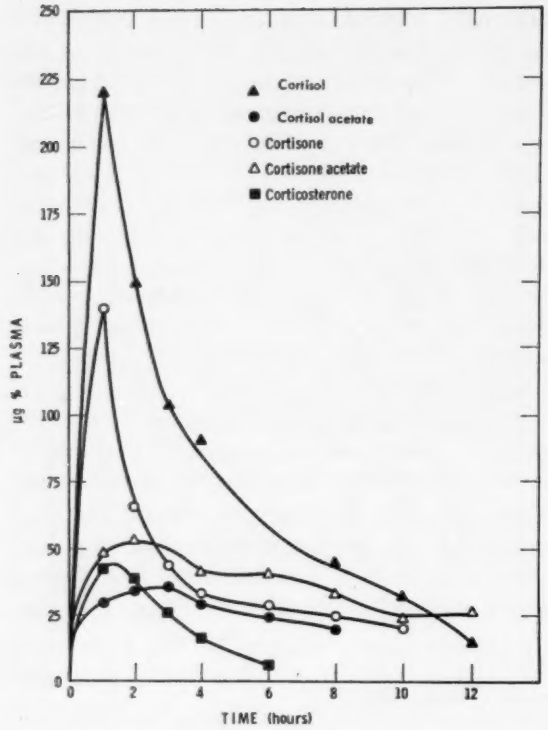

FIG. 7. COMPARATIVE PLASMA STEROID LEVELS FOLLOWING ORAL ADMINISTRATION OF 200 MG OF STEROIDS. CORTISOL AND CORTISOL ACETATE WERE DETERMINED AS PHENYLHYDRAZINE-REACTING CHROMOGENS. The steroid levels after cortisone and cortisone acetate represent cortisol as determined by the isotope dilution method. Corticosterone was determined by the isotope dilution method.

plasma levels of cortisol after the administration of cortisol and cortisone and their acetates. Following the administration of 200 mg of corticosterone acetate orally to this same subject, no increase in free corticosterone could be demonstrated in the plasma.

DISCUSSION

A wide range of pharmacological doses of infused corticosterone, like cortisol, disappeared rapidly from the plasma at a rate proportional to concentration. However, in the normal subjects corticosterone disappeared from the plasma at a slightly more rapid rate, with a biological half-time of 1.27 hours as compared to 1.83 hours for comparable doses of cortisol (19). The use of the modification (12) of the selective sulfuric acid-induced fluorescence assay of Sweat (22) made the measurements of the corticosterone concentrations in the plasma after infusion of corticosterone relatively simple. Under the conditions of the study this assay proved to be relatively specific. Its validity was evaluated by the use of the more selective isotope dilution assay (12), which usually yielded slightly lower plasma corticosterone levels and a slightly longer biological half-time for infused corticosterone. These differences probably indicated that some of the metabolites of corticosterone gave a moderate fluorescence and were metabolized more rapidly than corticosterone. The 20α and 20β reduced stereoisomers of corticosterone are known to be metabolites of corticosterone (31), and with the fluorescence assay they yield approximately one-third the fluorescent intensity of corticosterone (12). In one normal subject given 50 mg of the 20β reduced metabolite of corticosterone, the infused steroid disappeared from the plasma ($T_{1/2} = 0.9$ hour as determined by the fluorescence assay) more rapidly than corticosterone ($T_{1/2} = 1.3$ hours).

One difference between the curves of plasma steroid concentrations of the cortisol and corticosterone was that in the case of the latter the curve in some instances did not become linear until 30 to 90 minutes after the midpoint of the infusion, whereas with the former or with cortisone the curve was usually linear within 20 to 30 minutes (19, 20). This was true for both the fluorescence and the isotope dilution methods of assay, was characteristic of only a few individuals, and was not a function of the larger doses of administered corticosterone (50, 100 or 200 mg). This phenomenon was, however, shown to be dose-dependent, because with the rapid infusion of trace doses of either H^3-(7 to 30 μg) or C^{14}-(200 to 400 μg) labeled corticosterone, the decline in concentration of labeled corticosterone was always linear within 20 minutes.

In addition to the variations, in the nature of the curve of plasma corticosterone after trace and pharmacological doses, it was also noted that the slopes of the linear portions of the concentration curves were different at the two dose levels. With the trace dose the slope was steeper and thus yielded a shorter biological half-time. This was not found to be due to an altered metabolism of the labeled steroid. With 100 mg of labeled corticosterone the fluorometric assay showed a slope

similar to that obtained with the radioactive assay. In addition, the more rapid appearance and the higher plasma level of metabolites conjugated as glucuronides after the trace dose revealed another significant difference in the metabolism of corticosterone at different dose levels. Finally, determinations of the "apparent volume of distribution" of the labeled corticosterone at these two dosage levels also demonstrated a significant difference in the distribution of the infused labeled steroid (trace dose, 12 to 40 L; pharmacological dose, 60 to 90 L).

Except for the disparity in the initial portion of the curve of the declining corticosterone concentration at the two dosage levels, these same differences were noted with trace and pharmacological doses of cortisol (18). It seems likely that differences in the fraction of nonprotein-bound and protein-bound steroid at physiological (trace) and pharmacological levels may explain these discrepancies. On the basis of the recent reports on the binding of cortisol (32–36) and corticosterone (33–35, 37) to plasma proteins, it is evident that when a trace dose of the steroid is infused, close to 100 per cent of the circulating plasma steroid is protein-bound, whereas with pharmacological doses only 70 to 80 per cent is protein-bound. After the infusion of 20 to 200 mg of steroid, plasma steroid levels may range from 50 to 200 μg per 100 ml. Thus a larger fraction of nonprotein-bound steroid is available for diffusion into the extravascular space, and equilibration between plasma and the extravascular space may not be approximated rapidly. Under these circumstances the plasma concentration may not be expressed as a constant first order function but rather as a series of exponential terms in which the exponents are functions of the rate constants, for enzymatic transformation by the liver, diffusion into the extravascular space, and re-entry into the plasma. In the absence of significant excretion, even though the rate of decline in plasma concentration represents a first order process, the rate of disappearance of the steroid, especially after infusion of pharmacological quantities, may not always represent a reliable index of the rate of metabolism of endogenous steroid at physiological levels.

It has previously been reported that the chloroform-soluble radioactive metabolites of corticosterone-4-C^{14} injected in trace amounts disappeared more rapidly from the plasma than the radioactive metabolites of cortisol-4-C^{14} (17, 38). Also, the peak of the radioactivity of the glucuronide fraction in the plasma reached a maximum more rapidly. In one normal subject, two of the metabolites of corticosterone (20β-hydroxycorticosterone and 11-dehydrotetrahydrocorticosterone) and the 11α epimer of corticosterone were metabolized more rapidly than corticosterone. (20β-Hydroxycorticosterone and 11α-hydroxycorticosterone were assayed by the fluorescence method, and 11-dehydrotetrahydrocorticosterone by the double isotope dilution derivative assay.) Patients with liver disease showed no marked impairment in their capacity to metabolize corticosterone, whereas cortisol metabolism was significantly impaired (18, 39, 40). Patients with myxedema and thyrotoxicosis were shown to have an altered metabolism of cortisol (18, 41, 42), and a similarly altered corticosterone metabolism. Normal subjects treated with estrogens showed a delayed disappearance of both cortisol and corticosterone from the plasma (43). This has been related to alterations in concentration of the steroid-binding proteins after administration of estrogens (43, 44).

The major route of excretion of labeled metabolites of corticosterone, like that of cortisol and cortisone, was via the urine. Seventy to 90 per cent of the administered dose could be accounted for in the urine within three days. The cumulative rate of excretion of the labeled steroid metabolites was also similar for these three steroids—$T_{1/2} = 2.5$ to 4 hours. Only a small fraction of the metabolites of corticosterone was excreted into the gastrointestinal tract via the bile. Only 3 to 5 per cent of the administered steroid was excreted in the urine as radiometabolites directly extractable with dichloromethane, and less than 0.5 per cent of the administered corticosterone was recoverable as unaltered corticosterone. By paper chromatography the direct dichloromethane extracts of urines collected after infusions of 200 mg of corticosterone were found to contain trace amounts of the 20α and 20β hydroxy metabolites of corticosterone.[4] These findings were based on

[4] We are indebted to Dr. M. Darrach for a small supply of 20α-hydroxycorticosterone.

a comparison of the rates of migration of the authentic pure compounds, and by conversion of the steroids to the diacetates and comparison of their chromatographic behavior with that of the authentic derivatives. These two substances isolated from the urine also gave negative reactions with the phenylhydrazine and alkaline blue tetrazolium reagents. Both compounds also gave the alkaline and sulfuric acid fluorescence reactions. Glucuronidase hydrolysis released radiometabolites equivalent to 40 to 60 per cent of the administered steroid. Incubation of the urine at room temperature for 24 hours at pH 1 did not release any radiometabolites. These results are in agreement with the previous data reported for cortisol (19, 45–47), cortisone (20), and corticosterone (17, 38, 48).

Following the intravenous or oral administration of trace (200 μg) amounts of labeled corticosterone, comparable amounts of labeled metabolites were present in the urine within 24 hours. However, following the oral administration of 100 to 200 mg of the labeled steroid, a smaller fraction of the urinary metabolites appeared within 24 hours, and after feeding 100 mg of labeled corticosterone acetate, even less appeared in the 24 hour urine. These data are at variance with those on cortisol and cortisone, which indicated that after administration of the free alcohols of the steroids in trace or larger amounts the recovery of the radiometabolites in the urine was not appreciably altered (19, 20, 48). With cortisone acetate the oral absorption of trace and large doses also gave similar results. With trace doses of cortisol, cortisone and corticosterone and with their acetates, oral and intravenous administration gave comparable results. However, when administered in pharmacological amounts, cortisol acetate and corticosterone acetate and, to a lesser extent, corticosterone were poorly absorbed from the gastrointestinal tract. This was substantiated by measurements of the concentrations of cortisol and corticosterone in the peripheral blood after oral administration of 100 to 200 mg of the acetates, and the free alcohols of these steroids. After the administration of the acetates, only minimal increases of the plasma steroid levels were observed. After oral corticosterone the plasma levels were less than one-third those observed after administration of comparable amounts of cortisol. These lower plasma levels are probably the result of a less efficient absorption and more rapid metabolism of the corticosterone.

By utilizing a double isotope derivative or double isotope dilution assay, some information has been obtained regarding the identity and the quantities of the metabolites of corticosterone comprising the free and conjugated (glucuronide) fractions of the urine. In the normal subject the major α-ketolic steroids released after glucuronidase hydrolysis consisted of the tetrahydro (3α) and allotetrahydro (3α) metabolites of corticosterone and 11-dehydrocorticosterone. Allotetrahydrocorticosterone was the major metabolite, and the tetrahydrocorticosterone concentration usually exceeded that of 11-dehydrotetrahydrocorticosterone. Other investigators have also reported that the concentration of allotetrahydrocorticosterone exceeds that of other α-ketol tetrahydro compounds, and that the level of tetrahydrocorticosterone usually exceeds that of the 11-dehydro metabolites (49–52). Pregnane-3α,11β,20α-triol and 3α,11β,dihydroxypregnane-20-one have also been isolated from the urine of patients treated with large amounts of corticosterone (53). 11-Dehydrocorticosterone has also been isolated from the urines of such patients (52). Normally the predominant metabolite of cortisol is tetrahydrocortisone (3α), although tetrahydrocortisol (3α) and its 5α isomer, allotetrahydrocortisol (3α), appear in measurable quantities (54).

To resolve some of the uncertainties associated with estimations of corticosterone production from determinations of the α-ketol tetrahydrometabolites, we have utilized labeled corticosterone to obtain a more quantitative measure of the rate of synthesis of corticosterone. The same two techniques employed for cortisol turnover rates (18, 25, 27) were found applicable to corticosterone. The corticosterone-4-C^{14} available to us was, however, not of sufficiently high specific activity (0.004 μc per μg) to make possible determinations of the miscible pool size and pool turnover from kinetic measurements of the plasma specific activity of corticosterone-4-C^{14} after intravenous injection. Subsequent studies demonstrated that these measurements could not be made with anything approaching trace amounts of corticosterone-4-C^{14}. For this reason we used H^3-labeled corticosterone (1.5 μc per μg) prepared by the

Wilzbach technique (55). H^3-labeled steroids (17, 26, 56, 57), including cholesterol (58), have been used for tracer studies in human beings and in animals, and there is evidence that the H^3 label is sufficiently stable. We have previously published data on the metabolism of simultaneous intravenously administered mixtures of corticosterone-4-C^{14} and randomly labeled corticosterone-H^3 (18). In these studies H^3-labeled corticosterone and corticosterone-4-C^{14} did not appear to be metabolized differently. On the basis of the studies of Ayres and associates (17) it was indicated that H^3-labeled corticosterone (16-H^3) was metabolized like the parent steroid.

In the normal subjects given rapid infusions of trace quantities (7 to 30 μg) of corticosterone-H^3, the rapidly exchangeable pool of corticosterone ranged from 0.20 to 0.40 mg with a mean of 0.3 mg. In these same subjects the plasma concentration of corticosterone was 0.8 to 1.5 μg per 100 ml with a mean of 1.0 μg per 100 ml. From the mean plasma corticosterone concentration and the mean corticosterone pool size, it was possible to calculate on "apparent volume of distribution" of this pool. This was calculated to be 30 L. This volume, like that for cortisol (19, 25), has no physical reality and must be regarded solely as that theoretical volume required to contain the rapidly exchangeable corticosterone at its concentration in plasma. Following the infusion of 100 mg of unlabeled steroid, a two- to threefold larger volume of distribution was obtained, and this probably results from the fact that a larger fraction of the infused steroid diffused more rapidly into the extravascular space.

Within 8 to 12 hours after the continuous infusion of corticotropin, the miscible pool of corticosterone (and the plasma concentration) showed a four- to tenfold increase. This change in pool size exceeds that previously reported for cortisol after infusion of corticotropin (18, 25).

In the normal subjects both before and after the infusion of corticotropin the half-times of the specific activity curves of plasma corticosterone were similar as were the fractional rates of turnover of the miscible pool. However, after corticotropin the hourly rate of turnover of corticosterone was increased four- to tenfold.

With the kinetic method, it was possible to measure the rate of synthesis of corticosterone over a period of only a few hours because the labeled steroid could not be obtained with a sufficiently high specific activity so that a large enough quantity could be used to obtain a measurable yield of radioactivity and yet inject a trace quantity. Also, plasma corticosterone shows a diurnal variation similar to that of cortisol (13, 18), and thus the pool would probably not remain sensibly constant for more than a few hours. Studies of cortisol turnover at different times of the day revealed a marked variation in the hourly turnover rate (18, 25). By measuring the specific activity of one or more of the urinary radiometabolites of corticosterone, the daily rate of turnover of endogenous corticosterone was determined. With this method it was possible to use the lower specific activity corticosterone-4-C^{14}, since the 200 μg injected represented a trace quantity when compared to the total daily production. Results with either corticosterone-4-C^{14} or corticosterone-H^3 were in good agreement. The specific activities of tetrahydrocorticosterone, allotetrahydrocorticosterone and 11-dehydrotetrahydrocorticosterone were comparable, and thus it would appear that each of these metabolites arises solely from a common parent compound (corticosterone). With this technique rates of turnover of 1.3 to 4.0 mg per day were found for normal subjects. When the results of the kinetic method were expressed on the basis of the daily turnover, the data did not agree with the urine specific activity method, and in all instances the former method gave higher values. This undoubtedly indicates that the rate of turnover of corticosterone during the morning hours, at a time when the plasma level of corticosterone is highest (13), is greater than it is at other times of the day. Inaccuracies in the determination of the pool size may also be responsible for appreciable errors in the kinetic assay. Since the corticosterone is metabolized rapidly, prompt dilution of the labeled steroid within the pool, prior to its metabolism, may not occur, and thus the pool size calculations represent only a first approximation. Also, other slower mixing pools may be present and undetected with this type of measurement.

In the studies with corticotropin the turnover rates by the kinetic method, when extrapolated to 24 hours, agreed reasonably well with the results obtained from the urine specific activity method.

In these instances, however, the miscible pool of corticosterone probably remained reasonably constant. In the bilaterally adrenalectomized subject, the specific activity of the labeled urinary tetrahydrocorticosterone was close to that of the injected labeled corticosterone, indicating no significantly measurable secretion of corticosterone, or other extra-adrenal products metabolized to the tetrahydro metabolites of corticosterone.

In the only other study of corticosterone secretion, Ayres and co-workers (17) reported a daily turnover of 0.84 mg in one normal subject. They measured the dilution of H^3-labeled corticosterone by assaying the specific activity of the urinary corticosterone. In two of the subjects of this study the daily turnover rate was calculated by measuring the specific activity of the urinary corticosterone, and a lower secretion rate was found (one-third to one-half that obtained by assay of the tetrahydrometabolites). We also observed this discrepancy between the specific activities of the cortisol and the tetrahydro metabolites of cortisol in normal subjects (18). Cope and Black (27) also observed this difference, i.e., a higher specific activity for the urinary cortisol. It seems probable that these differences may result from the fact that when the steroid is injected rapidly (three to five minutes), it is not well mixed with the body pool prior to excretion, and a larger amount is excreted initially as the free compound.

It is of interest that with both cortisol and corticosterone the fraction of the steroids excreted as the 3α tetrahydro and 3α allotetrahydro metabolites conjugated as glucuronides represented one-third to one-half of the total daily production of the steroids. This was also noted after administration of corticotropin or 100 mg of the steroids. Normally the tetrahydro metabolites of corticosterone are present in the urine in much lower concentrations than the tetrahydro metabolites of cortisol.

Peripheral plasma values of corticosterone have in many instances been reported to approximate or equal those for cortisol (59). This suggested that adrenal corticosterone secretion approached that of cortisol. Bush has reviewed this information (60) and has suggested that the higher plasma corticosterone values previously reported probably resulted from inadequate purification of the steroid prior to analysis. The data on the relative amounts of cortisol to corticosterone in the miscible pool, in the peripheral blood (9–16), and in urine (13, 17) support the concept that corticosterone secretion is very much less than that of cortisol and the turnover rate studies confirm this.[5] Other than the fact that corticosterone is metabolized slightly more rapidly than cortisol, the physiological disposition and metabolic fate of these two steroids are not greatly different.

SUMMARY

In normal subjects infused corticosterone was shown to disappear from the plasma more rapidly than cortisol. With the administration of large (therapeutic) doses of corticosterone, its rate of disappearance was found to be proportional to concentration. However, trace doses of labeled corticosterone disappeared from the plasma more rapidly than the larger doses. In patients with cirrhosis and myxedema the disappearance of corticosterone from the plasma was only slightly slower than normal, whereas in normal subjects treated with estrogens its disappearance was definitely delayed. In patients with thyrotoxicosis the steroid disappeared from the plasma very rapidly.

Normal subjects within 72 hours excreted 80 to 90 per cent of the administered hormone as urinary and biliary metabolites, as judged by urinary (70 to 80 per cent) and biliary (11 per cent) excretion of C^{14}- or H^3-labeled steroids. Approximately 3 per cent of the administered hormone was excreted unconjugated, and less than 0.5 per cent as unchanged corticosterone. Forty to 60 per cent of the administered dose was excreted as metabolites hydrolyzed with β-glucuronidase. This fraction consisted predominantly of the tetrahydro and allotetrahydro reduction products of corticosterone. Allotetrahydrocorticosterone was the major tetrahydro metabolite. When administered in pharmacological doses, corticosterone, and especially corticosterone acetate, were found to be poorly absorbed from the gastrointestinal tract.

Utilizing trace quantities of intravenously ad-

[5] Human adrenocortical tissue has also been found to contain less corticosterone than cortisol (4, 61). Adrenal cortical tissue slices on incubation produce less corticosterone than cortisol (61). Also, human adrenal vein plasma contains one-fifth to one-tenth as much corticosterone as cortisol (61).

ministered corticosterone-H^3, the miscible pool size of corticosterone was found to be 0.2 to 0.4 mg, and the fractional turnover of the pool 0.7 mg per hour. Calculations of the rate of turnover of endogenously synthesized corticosterone, by measurements of the dilution of the specific activity of the urinary tetrahydro metabolites of intravenously administered corticosterone-4-C^{14} or corticosterone-H^3, indicated a daily rate of production of 1.5 to 4.0 mg. Corticotropin increased the rate of synthesis of corticosterone seven- to tenfold.

Although the physiological disposition and metabolic fate of cortisol and corticosterone were not greatly different, the quantity of corticosterone secreted by the adrenal was only one-fifth to one-tenth that of cortisol. This results in a lower circulating plasma corticosterone level, and the excretion of a smaller quantity of the tetrahydro metabolites of corticosterone.

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EXCRETION OF CATECHOLAMINES, 17-KETOSTEROIDS, 17-HYDROXY-CORTICOIDS AND 5-HYDROXYINDOLE IN MEN EXHIBITING A PARTICULAR BEHAVIOR PATTERN (A) ASSOCIATED WITH HIGH INCIDENCE OF CLINICAL CORONARY ARTERY DISEASE * †

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Several recent studies from this laboratory strongly suggest that a particular overt behavior pattern may be strongly implicated in the pathogenesis of many cases of clinical coronary artery disease. Thus it was observed (1) that when accountants were forced by occupational circumstances to alter acutely their usual behavior pattern of relative calm to an overt behavior pattern in which competitive and excessive drive and an enhanced sense of time urgency were paramount, a marked elevation of serum cholesterol and hastening of blood clotting time occurred. It is of interest that this observed rapid rise of serum cholesterol following change in behavior pattern already has been confirmed by three different groups of investigators (2–4). It was further found (5) that a group of individuals who chronically exhibited an overt behavior pattern (Pattern A) characterized by excessive and competitive drive and an enhanced sense of time urgency not only exhibited notably higher serum cholesterol and faster clotting time than individuals exhibiting a converse type of behavior pattern (Pattern B), but also an approximately sevenfold higher incidence of *clinical* coronary artery disease and over three times as much arcus senilis. Finally, we have more recently found (6) that females exhibiting this same Behavior Pattern A exhibit approximately the above same differences in blood and cardiovascular findings, when compared to females showing Pattern B. These women with Pattern A appeared to be remarkably similar to men with Pattern A as regards the incidence and intensity of their blood and cardiovascular abnormalities.

Since the dietary, physical activity and various other characteristics of these different types of individuals were approximately the same, we concluded that in some manner Behavior Pattern A itself was causally responsible for the altered lipid and clotting dynamics of the blood and for the clinical changes found. The question of how such a behavior pattern might induce such changes remained to be clarified. As a first attempt to ferret out possible clues, it was decided to study the urinary excretion of some possibly relevant hormones in groups of men exhibiting Behavior Pattern A or Pattern B. The results of this study are presented below.

METHODS

Choice of men and method of urine collection. In our earlier study (2) a large group of men had been chosen for us (by lay selectors) who exhibited either Behavior Pattern A or B. In our own personal interview, we also had the opportunity to judge for ourselves the intensity of the pattern present in each volunteer subject. We therefore selected 12 men (between the ages of 35 and 50 years) from each of these two groups who we believed possessed *most completely* the characteristics of the respective two behavior patterns.

Earlier we expressed our belief (5) that Behavior Pattern A might be a resultant of the interplay between a provoking environment and a particular type of personality. We therefore employed three general criteria in selecting the 12 men of Group A. First, each of the 12 men occupied a position not necessarily of top executive quality but associated with or demanding extreme competitive activity and deadline preoccupations. Thus, of the 12 men selected, 4 were managing or city editors; 7 were corporation executives who were themselves

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directly engaged in highly competitive merchandising or engineering activities; and one was an advertising account executive supervising a particularly difficult account.

Second, each of the men chosen admitted the chronic presence of a rather marked sense of competitive drive and most particularly a severe sense of time urgency.

Third, and most important, all of the men had exhibited various motor phenomena during our interview with them suggesting the presence of a severe sense of time urgency. These phenomena were *1)* a rapid, frequently forced and audible intake of breath, *2)* explosive intonation or emphasis of individual words of sentences, *3)* tense facial and body musculature, *4)* frequent fist clenching accompanying ordinary conversation, *5)* consistent tendency to hasten the pace of ordinary conversation either by attempting to finish the sentences of the interviewer or to hasten the latter's rate of speaking by uttering rapidly "yes, yes" or "I see, I see" even before the interviewer had finished his sentence.

The converse of these three criteria was used to select 12 men having the B Pattern. Their positions demanded neither competitive activity nor preoccupation with deadlines. Thus of the 11 selected men who agreed to participate, 8 were municipal employees performing clerical or accounting duties; 2 were employee embalmers; and one was an employee public accountant.

None of the men of either group was ever aware of suffering from *symptomatic* clinical coronary artery disease. All were normotensive.

If a particular behavior pattern might alter the secretion, hence also the excretion rate of hormones to be studied, obviously the products of excretion should be collected at the time that the particular behavior pattern might be most expected to be present. Each subject, therefore, was requested to collect all of his urine while at work. We obtained approximately half of the day's sample from 10:00 a.m. to 12:00 noon and the remainder from 2:00 to 5:00 p.m. Urine voided on reaching work at 9:00 a.m. and after returning from lunch at 2:00 p.m. was discarded. Urine was thus collected separately from each subject on each of four successive working days (Monday to Thursday, inclusive), but then was pooled and analyzed as described below. In addition, a single overnight urine sample was obtained from each subject immediately upon arising. We thus were able to obtain urine formed during the height of their working period and also during complete bed rest. Each man also was asked to estimate the intensity of his sense of urgency during the four days of urine collection. The men were cautioned not to ingest bananas during the four day period of urine collection. None was taking any type of medication. The urines of four men (two of Group A and two of Group B) were obtained each week.

Urinary assay of 17-ketosteroids, 17-hydroxycorticosteroids, 5-hydroxyindole, epinephrine and norepinephrine. In all of the chemical assays listed below, a urine from a man of Group A was always analyzed in the same run with a urine obtained from a man of Group B.

The creatinine content of each four day pooled and single night specimen of urine was determined by the method of Bonsnes and Taussky (7). The 17-ketosteroid content of the same specimens was determined according to the method of Klendshoj, Feldstein and Sprague (8). The 17-hydroxycorticosteroid analysis was made according to the method of Silber and Porter (9). The 5-hydroxyindole analysis was done according to the method of Dalgliesh (10). The epinephrine and norepinephrine content of the urine samples was determined according to the method of Sobel and Henry (11), as modified by Forsham (12). This modification consisted of the addition of 1 ml of 2 per cent ascorbic acid and 1.5 ml of 1 per cent ethylenediaminetetraacetate disodium to 15 ml of hydrolyzed urine aliquot just prior to adjusting pH to 8.4 and chromatographing on Al_2O_3. Aliquots of the acetic acid eluate were oxidized with ferricyanide at pH 3.5 and 6.0 and stabilized with additional alkaline ascorbate. In our hands, the standard deviation of individual values in replicate analyses was ± 8 per cent for norepinephrine and ± 10 per cent for epinephrine. Recovery of added epinephrine ranged between 90 and 94 per cent, and of added norepinephrine, between 88 and 94 per cent.

The concentrations of these substances were expressed in terms of milligrams or micrograms per gram of creatinine, for comparative purposes.

Study of dietary, tobacco, alcohol, work and exercise habits. These data already had been obtained in these men in 1958 (2), and as far as we could determine there had been no change of any consequence in their habits except that one individual (W.L. of Group A) had given up smoking cigarettes.

Study of serum cholesterol, blood clotting, and incidence of arcus senilis and clinical coronary artery disease. These data also had been obtained in these men in 1958 (2). Serum cholesterol determinations were repeated in 6 of the 23 men, and as Table I indicates, there was no essential change from the values obtained in these same men in 1958.

RESULTS

General characteristics of the two groups. As Table I indicates, the age, weight and height of the two groups of men were approximately the same. The hours of working and the average amount of physical activity were greater in men of Group A. However, the average excess physical activity of the men of Group A was due to their indulgence in weekend sports. During the weekday period of urine collection, however, these men indulged in no more, but probably in less, physical activity than the men of Group B. Once again (2) the cigarette and alcohol consumption were found to be considerably greater in men of this same group.

TABLE I

Summary of physical, dietary, serum cholesterol and cardiovascular data of men of Groups A and B

Subject	Age	Ht.	Wt.	Physical activity: Work	Physical activity:	Cigs.	Alcohol	Total calories	Protein	Carbo.	Fat	Animal fat	Fat: per cent of total caloric intake	Positive parent. history	Arcus senilis	Clinical coronary disease	Serum cholesterol level*	Clotting time
	yrs	*in.*	*lbs*	*hrs/wk*	*hrs/wk*	*no./day*	*cal/day*										*mg/100 ml*	*min*
Men with Behavior Pattern A																		
D.S.	46	72	210	50	15	0	0	2,165	305	956	904	578	41.5	no	no	yes	245	5.3
D.B.	49	69	164	50	14	0	591	2,598	431	828	1,338	1,080	49.6	no	yes	no	241	6.2
A.W.	45	70	180	50	8	60	0	1,753	390	579	784	543	44.5	yes	yes	no	300 (310)	6.2
J.G.	35	74	205	45	4	30	155	2,731	376	969	1,386	950	50.8	yes	yes	no	244	7.0
A.M.	46	68	147	50	1	60	330	1,927	308	646	973	591	50.5	yes	no	no	300 (294)	6.8
C.L.	47	71	193	55	16	80	462	1,705	257	513	935	820	55.0	no	yes	yes	233	6.6
A.K.	43	72	163	50	20	40								no	yes	yes	272	7.0
R.L.	42	71	150	40	8	30	36	2,592	465	861	1,266	972	48.0	yes	no	no	301	5.8
C.S.	42	67	170	90	4	10	356	1,506	231	489	786	588	52.0	no	yes	no	366	6.2
W.O.	50	74	183	44	5	20	233	2,339	258	786	1,295	784	55.4	no	yes	yes	232	7.0
W.L.	44	70	165	60	14	0	303	1,937	257	743	937	769	48.4	no	yes	no	236 (238)	6.8
F.H.	44	75	165	66	16	20	90	1,601	229	642	730	503	45.5	no	no	no	341 (348)	
Average	45	71	175	54	10	21	232	2,078	319	728	1,030	743	49.0				276	6.4
SE of mean						±7.0	±56	±126	±24	±43	±98	±52	±1.3				±12.4	±0.16
Men with Behavior Pattern B																		
W.M.	50	67	186	40	12	0	40	1,381	295	362	724	604	52.5	no	no	no	196 (188)	7.0
A.R.	43	69	160	40	7	0	0	2,470	349	1,127	994	643	40.2	no	no	no	209	7.9
S.G.	50	75	197	40	6	0	0	2,538	428	882	1,228	841	48.5	yes	no	no	246	7.2
H.B.	45	65	150	40	10	0	0	1,258	232	500	526	452	42.0	no	no	no	179	7.2
C.M.	43	69	235	40	2	4	20	2,906	329	1,248	1,319	658	40.5	no	no	no	234	7.0
A.M.	43	72	149	40	14	40	0	3,446	591	711	2,144	1,231	62.0	no	no	no	168	7.4
O.J.	44	73	165	40	4	20	341	1,794	296	590	908	637	50.5	no	no	no	217	7.4
E.G.	50	71	180	40	2	50	70	1,452	238	513	701		48.5	no	no	no	236	6.4
G.M.	40	68	172	50	3	25	105	1,950	200	840	910		47.0	no	no	no	170	8.4
H.L.	45	70	175	40	3	20	136	1,885	269	783	833	579	44.0	no	no	no	216	6.2
F.A.	50	69	155	48	7	0	155	2,234	300	814	1,120	739	50.5	yes	no	no	230 (250)	7.5
Average	45	70	174	42	6	14	79	2,119	321	761	1,037	712	48.0				209	7.2
SE of mean							±30	±190	±32	±75	±132	±68	±1.8				±9.7	±0.18

* Values in parentheses represent repeat serum cholesterol analyses obtained in 1959.

TABLE II

*The renal excretion of various hormones in men of Groups A and B**

Subject	Pooled day time urine							Single night urine					
	Creat.	17-KS	17-HOCS	5-HI	Epineph.	Norepineph.	T.C.	Creat.	17-HOCS	5-HI	Epineph.	Norepineph.	T.C.
	mg/ml	*mg/g creat.*	*mg/g creat.*	*mg/g creat.*	*μg/g creat.*	*μg/g creat.*	*μg/g creat.*	*mg/ml*	*mg/g creat.*	*mg/g creat.*	*μg/g creat.*	*μg/g creat.*	*μg/g creat.*
Men with Behavior Pattern A													
D.S.	1.68	5.9	4.3	2.56	13.7	33.4	47.1						
D.B.	1.07	7.5	4.2	2.30	24.2	29.0	53.0	2.13	1.7	1.4	11.6	14.2	25.8
A.W.	1.25	7.2		2.60	28.8	34.8	63.6	1.37	3.2	2.3	9.5	16.7	26.2
J.G.	1.41	9.0	3.3	2.70	15.0	38.0	53.0	1.66	1.3	3.5	2.4	18.1	20.5
A.M.	1.67	9.0	2.7	1.80	25.0	50.0	75.0	1.85	1.1	2.5	7.6	13.0	20.6
C.L.	1.37	7.1	3.1	2.5	25.0	48.0	73.0	1.11	2.7	2.9	12.6	23.4	26.0
A.K.	1.25	4.8	2.3	1.7	33.0	53.0	86.0	1.44	1.9	1.7	43.5	0	43.5
R.L.	2.22	3.4	1.8	1.7	26.7	45.6	72.3	1.43	2.2	2.1	11.7	15.8	27.5
C.S.	0.84	7.4	3.8	2.2	20.0	31.0	51.0	2.56	1.5	2.8	12.5	13.3	25.8
W.O.	2.23	14.2	2.8	2.2	38.5	33.6	72.1	1.00	1.6	2.5	9.5	23.0	32.5
W.L.	0.80	7.5	6.7	2.1	32.5	29.0	61.5	2.07	3.2	1.9	17.0	11.6	28.6
F.H.	1.13	8.9	6.5	3.6	39.0	21.0	60.0	2.25	2.1	3.1	20.0	3.5	23.5
Average	1.41	7.6	3.7	2.3	26.8	38.0	64.0	1.72	2.0	2.4	14.4	13.9	27.3
SE of Mean	±0.17	±0.8	±0.4	±0.14	±2.3	±2.4	±3.3	±0.2	±0.2	±0.2	±3.1	±1.8	±1.8
Men with Behavior Pattern B													
W.M.	1.14	8.8	5.4	2.40	26.0	22.0	48.0						
A.R.	1.14	5.7	5.3	1.58	21.0	23.0	44.0						
S.G.	1.18	6.8	4.1	3.5	14.4	34.7	49.1	1.33	1.8	3.3	11.2	29.3	40.5
H.B.	1.20	16.7	4.4	2.2	10.8	15.0	25.8	1.95	3.5	4.5	5.7	8.8	14.5
C.M.	2.4	10.2	2.7	2.1	21.6	26.6	48.2	1.99	1.8	1.5	9.2	14.9	24.1
A.M.	1.63	9.8	2.0	2.2	17.0	35.0	52.0	1.56	2.3	2.1	29.0	13.6	42.6
O. J.	1.50	6.7	3.7	2.5	26.6	15.3	41.9	0.60	1.0	2.0	7.6	9.6	17.2
E.G.	1.69	7.1	1.9	2.4	47.0		47.0	2.42	1.4	2.5	21.0	16.0	37.0
G.M.	1.60	7.5	2.1	2.2	25.0	9.4	34.4						
H.L.	1.70	6.1	4.1	2.2	26.5	27.0	53.5	2.9	2.7	2.6	30.0	0	30.0
F.A.	1.66	5.8	2.9	2.5	20.5	30.0	50.5	1.37	1.7	2.7	23.0	13.6	36.6
Average	1.53	8.3	3.5	2.3	23.3	21.6	44.9	1.41	2.0	2.7	17.1	13.2	30.3
SE of mean	±0.1		±0.1	±0.12	±7.5	±3.1	±2.4	±0.3	±0.3	±0.3	±3.3	±2.5	±4.2

* 17-KS = 17-ketosteroids; 17-HOCS = 17-hydroxycorticoids; 5-HI = 5-hydroxyindole; T.C. = total catecholamine.

The dietary habits of the two groups. The intake of total calories, protein, carbohydrate and fat (also animal fat) was essentially the same in both groups (see Table I). Similarly the per cent of the total diet ingested as fat was approximately the same.

Serum cholesterol and clotting time of the two groups. As Table I shows, despite the small number of subjects involved, the average serum cholesterol of the men of Group A (276 mg per 100 ml) is remarkably and significantly higher than that of Group B. Again, the clotting time is significantly faster in the men of Group A than in the men of Group B. These average differences in serum cholesterol and blood clotting time were greater than those noted in the earlier study because, we believe, the extreme examples of both types of behavior pattern were chosen for the present study.

The incidence of arcus senilis and clinical coronary artery disease. The previously observed striking differences in incidence of arcus senilis and clinical coronary disease again were found. Thus 8 of the 12 men of Group A, but none of the men of Group B, displayed an arcus senilis. Similarly, four of the men of Group A and none of Group B were found to have clinical, but asymptomatic coronary artery disease. The evidence for the latter's presence was electrocardiographic in each case. Two of the four electrocardiograms, besides exhibiting characteristic patterns of myocardial damage, also indicated the occurrence of old infarcts that had escaped clinical recognition.

The renal excretion of various hormones in the two groups. Eleven of the 12 men of Group A reported that the pooled daytime urine was collected while they were feeling a moderate to severe sense of urgency doing their job. On the other hand, 7 of the 11 men of Group B reported feeling no sense of urgency during the daytime urine collection. The remaining four felt only a slight to moderate sense of urgency. It was interesting that the renal excretion of the various hormones studied was approximately the same (see Table II) when both groups were at bed rest. However, quite significant differences appeared during the working periods of the two groups.

In both groups, the excretion of 17-hydroxycorticoids increased comparably during the working periods. This observed higher daytime excretion of 17-hydroxycorticoids in almost all the subjects confirms the earlier observation of Laidlaw, Jenkins, Reddy and Jakobson (13). This finding also suggests that each of the subjects had carefully followed our directions for the separate collections of the two types of urine. The urinary excretion of 17-ketosteroids during the working period also was approximately the same in the two groups. The excretion of 5-hydroxyindole also was found to be approximately equal both during the night and during working periods in the two groups.

A marked increase in the excretion of epinephrine occurred during the working period of both groups. Although an increase of 86 per cent occurred in the men of Group A and 36 per cent in the men of Group B, the difference was not statistically significant. However, the increase in the excretion of norepinephrine during the day (173 per cent) shown by the men of Group A was of much greater magnitude than the increase (64 per cent) observed in the men of Group B and this difference was significantly different (difference of means = 4.2 × SE difference of means). The daytime excretion of the total unchanged catecholamines by the men of Group A also was significantly greater than that by the men of Group B (difference of means = 4.7 × SE difference of means).

As Table II indicates, no correlation was found between the average weekly amount of physical activity done by men of either group and their norepinephrine secretion. This absence of correlation we believe was due to the fact that the greater amount of physical activity reported by a number of individuals was due not to the demands of their job but to the propensity of these individuals to indulge in sports during the weekend —a period in which the urine collections were not obtained.

DISCUSSION

Earlier (2) we stressed our belief that Behavior Pattern A was a resultant of the interaction of two variables, namely, the specific reaction of an individual to his given environment, and the quality and intensity of the inciting or provocative environment itself. So composed, a change

either in the individual's reaction (endogenous) or in the inciting environment (exogenous) easily could modify or even conceivably eliminate the pattern.

Because of this, we felt that any study of the possible biochemical consequents of Behavior Pattern A should be conducted precisely during the individual's exposure to that milieu most likely to evoke it. The present study indicates rather clearly, we believe, that when such precautions were taken, individuals exhibiting Pattern A excreted far more norepinephrine than subjects exhibiting the converse pattern. However, when the specific milieu was not provoking (i.e., bed rest), the catechol excretion of both groups of men was essentially identical.

These findings suggest that the excess catechol excretion by the men exhibiting Pattern A is not a fixed but rather a phasic phenomenon apparent only when they are studied in the appropriate environment. Indeed, if only 24 hour urine collections had been made, we seriously doubt if the differences found during the working period would have been sufficiently great to offset the probable equivalence of values obtained during the remaining 16 to 18 hours of the total 24 hour period. We stress these matters because if the present results are to be confirmed, the urine must be collected when each type of behavior pattern is in operation.

A good illustration perhaps of the importance of securing the proper conditions for the study of catecholamine discharge was the finding of Raab and Gigee (14, 15), confirmed later by Gazes, Richardson and Woods (16), of a marked increase in the plasma catecholamine of patients with angina after exercise. Yet both groups of authors were unable to observe a similar increase of catecholamine either in the urine or in the plasma of patients convalescing from acute infarction *while they were at bed rest.* Indeed, it is unlikely that patients who had suffered from a clinically recognized infarction would later exhibit excess discharge of catecholamine since such patients, whether of their own volition or guided by medical advice, often radically alter either those facets of their own personality or those particular environmental factors, the preinfarction combination of which may have induced Behavior Pattern A. Certainly we do not believe that this behavior pattern is an unalterable, psychophysiological status.

Can a *phasic* excess discharge of catecholamines occurring day in and day out over many years be responsible for the lipid, clotting and cardiovascular abnormalities to which men exhibiting Pattern A are so prone (1, 5)? Or is this phenomenon too, as are possibly the serum cholesterol elevation, the excessive cigarette smoking and alcohol ingestion, only a congener of the pattern, bearing seemingly (5) little relevance to the pathogenesis of the clinical coronary artery disease so frequently found in men with this pattern? We strongly suspect that this excess catecholamine discharge may play more than such an associative role, for the following reasons. First, the marked increase in incidence of arcus senilis and coronary artery disease previously found (5) in men with Behavior Pattern A could not be ascribed to any excess ingestion of fat, tobacco or alcohol, or solely to the elevated serum cholesterol also observed in men with this pattern. Second, the behavior pattern has those qualities of excessive competitive drive and time urgency already shown by others (17, 18), as well as by us, to induce excess catecholamine discharge. Such behavioral characteristics, moreover, are not found in patients with anxiety neurosis, nor have the latter been found to excrete excess catecholamines (19). Third, excess catecholamine has been shown to be capable of altering lipid dynamics (20–23) and perhaps in the direction in which they were observed in men with Behavior Pattern A. Finally, administration of catecholamine markedly worsens the severity of the atherosclerosis occurring in laboratory animals fed excess cholesterol (24, 25).

Tempting as the above hypothesis may be, it must tentatively remain only a hypothesis. It would appear, however, that the present study has clearly demonstrated at least one biochemical pathway by which a psychological process may enter into and possibly influence man's more tangible, hence measurable, metabolic functions. It would now appear even more important that certain psychological processes and their resultant biochemical changes not be neglected in future studies of human coronary artery disease, as has so frequently been done in the past.

SUMMARY

The urinary excretion of 17-ketosteroids, 17-hydroxycorticosteroids, 5-hydroxyindole, epinephrine and norepinephrine was measured in a group of 12 men exhibiting an overt behavior pattern (A) found in earlier studies to be associated with a high incidence of clinical coronary artery disease. The nocturnal urinary excretion of these hormones was found to be the same in these men as in control men exhibiting a converse behavior pattern. However, when urine formed during working hours was analyzed, it was found that a far greater increase in norepinephrine occurred in the urine of the men exhibiting Pattern A than in the control groups. The possible relevance of these findings to the pathogenesis of clinical coronary disease was discussed.

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THE ROLE OF THE ANTERIOR PITUITARY IN THE CONTROL OF ALDOSTERONE SECRETION IN EXPERIMENTAL SECONDARY HYPERALDOSTERONISM *

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Previous reports (1–9) have delegated a secondary role to anterior pituitary hormones in the control of aldosterone secretion.[1] Although hypophysectomy resulted in decreased aldosterone secretion (3) or reduced urinary aldosterone output (2), hyperaldosteronuria, marked sodium (Na) retention and ascites occurred in the absence of the adenohypophysis in dogs with thoracic inferior vena cava constriction (2). Also, in patients with hypopituitarism, Luetscher and Axelrad (6) and Hernando and associates (7) found that urinary aldosterone output was within normal limits in some patients on an unselected diet or a normal salt intake, and Liddle, Duncan and Bartter (8) reported hyperaldosteronuria in one patient with hypopituitarism on a low Na diet.

However, no conclusive evidence of hypersecretion [1] of aldosterone in the absence of anterior pituitary hormones has been reported. The critical pertinent data on the rate of aldosterone secretion during stimulation which produces hypersecretion in normal animals have not been reported for hypophysectomized dogs or patients. It is important that studies in man be conducted on hypophysectomized patients since patients with so-called panhypopituitarism may not have loss of all anterior pituitary function. Furthermore, our knowledge is incomplete on the importance of specific anterior pituitary hormones. Several studies (3, 7, 8–10) have demonstrated an increase in aldosterone secretion or urinary aldosterone excretion following administration of various corticotropin preparations, but the data are inadequate to establish the role of ACTH in secondary hyperaldosteronism.[2]

The question of the role of the anterior pituitary in the control of aldosterone secretion was reopened by the finding of a 76 to 97 per cent fall in adrenal vein aldosterone output following hypophysectomy of dogs with experimental secondary hyperaldosteronism (11). In the present report, data are presented on the efficacy of ACTH [3] in preventing this fall in aldosterone secretion which follows hypophysectomy. Large doses of cortisone have been administered to inhibit ACTH secretion in dogs with hyperaldosteronism secondary to caval constriction; the resultant effects on aldosterone and corticosterone production were observed. Subsequently, the effects of hypophysectomy and ACTH were studied in these animals. Also, the effects of synthetic α-melanophore-stimulating hormone (MSH) and of highly purified preparations of natural α- and β-MSH have been studied. Attempts have been made to stimulate hypersecretion of aldosterone in simple hypophysectomized dogs by *1*) a low Na diet and *2*) acute constriction of the thoracic inferior vena cava.

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[1] The phrases "aldosterone secretion" or "hypersecretion of aldosterone" have been used to refer to actual measurements of the rate of secretion by the adrenal gland into the effluent plasma and are to be distinguished from urinary aldosterone excretion.

[2] In this paper the trivial names of the steroids have been used: cortisone (17α,21-dihydroxy-4-pregnene-3,11,-20-trione); corticosterone (11β,21-dihydroxy-4-pregnene-3,20-dione); 11-dehydrocorticosterone (21-hydroxy-4-pregnene-3,11,20-trione); aldosterone (11β,21-dihydroxy-18-aldo-4-pregnene-3,20-dione); cortisol (11β,17α,21-trihydroxy-4-pregnene-3,20-dione); and 2-methyl cortisol (2α-methyl-11β,17α,21-trihydroxy-4-pregnene-3,20-dione).

[3] Subsequent reference will be made to ACTH or to an ACTH preparation; it is recognized that contaminating substances with ACTH-like activity may be present.

METHODS

The 26 animals used in this study were 15 to 20 kg mongrel dogs. Nine animals were dogs with chronic experimental hyperaldosteronism and ascites secondary to constriction of the thoracic inferior vena cava (12); urinary Na excretion was less than 5 mEq per day on a Na intake of 60 mEq per day. The other 17 animals were simple hypophysectomized dogs at the onset of the experiment. In Experiment 1, the efficacy of ACTH in blocking the usual striking fall in aldosterone and corticosterone secretion which follows hypophysectomy was studied. In Experiment 2, large daily intramuscular doses of cortisone acetate (100 mg to 4 dogs and 200 mg to 3 dogs) were given for 8 days except that in the 3 animals receiving 200 mg daily, 100 mg of the cortisone was given orally on the last day. On the last day of cortisone administration, adrenal vein aldosterone and corticosterone output was measured. Five of the 7 dogs receiving cortisone were hypophysectomized after the 3 initial observations on steroid secretion; subsequently, in 4 of these 5 dogs the effects of ACTH were evaluated. In Experiment 3, preparations of α- and β-MSH were assayed in 4 simple hypophysectomized dogs. In Experiment 4, 10 simple hypophysectomized dogs were placed on a low Na diet for 7 to 19 days; for 5 of the dogs, Na intake was 7 mEq per day, whereas the other 5 animals received 1 mEq per day of Na. Potassium intake was 40 mEq per day. After acute adrenal vein aldosterone and corticosterone measurements in these hypophysectomized dogs on a low Na intake, the effects of ACTH were studied in 4 animals. In 3 additional dogs, the anterior pituitary was removed and after 2 hours the thoracic inferior vena cava was constricted acutely in an attempt to stimulate the adrenal cortex.

The same dose and preparation of ACTH were used in all experiments; 0.075 unit per minute of an Upjohn preparation was given intravenously in 5 per cent glucose at a rate of 0.62 ml per minute of solution with a constant infusion pump into the left subclavian vein. Synthetic α-MSH was prepared and supplied to us by Dr. Klaus Hofmann. This synthetic preparation is like the natural hormone except that glutamic acid is in the form of glutamine, and lysine is in the form of ϵ-formyllysine. The synthetic preparation was assayed by Dr. Aaron B. Lerner and found to have 6.6×10^6 units of MSH activity per mg. Highly purified preparations of α- and β-MSH from hog anterior pituitary were provided by Doctors Aaron B. Lerner and Teh H. Lee. The MSH potency of the natural α-MSH preparation was 2.8×10^6 units per mg, whereas the β-MSH preparation had 2.6×10^6 units of activity per mg. The MSH preparations were dissolved in 5 per cent glucose and infused at the rate of 0.62 ml per minute over a 30 minute period.

Observations on aldosterone and corticosterone secretion in adrenal vein plasma were made at 30 to 60 minute intervals in animals under light Nembutal anesthesia. The dogs received 100 per cent oxygen through an endotracheal tube attached to a pneophore at a pressure of 10 to 15 cm water. The right adrenolumbar vein was cannulated and measurements of adrenal blood flow were made by techniques described previously (13). Ten or 20 ml of adrenal vein blood and 10 ml of peripheral blood were removed for each group of analyses; normal donor blood was injected immediately to replace the blood removed for analysis. Urinary Na was measured by flame photometry. Cardiovascular pressures were measured in the abdominal aorta and inferior vena cava with Statham strain gages and a Sanborn recording system. An infusion of norepinephrine (100 μg per ml) was given to 3 of the hypophysectomized dogs on a low Na diet (Dogs 4, 6 and 8) during the latter part of the experiment to maintain arterial pressure and adrenal blood flow.

The concentrations of aldosterone and corticosterone in adrenal vein plasma were measured by the double isotope derivative procedure as described previously (14), with one exception; a third chromatography was used to purify corticosterone. Corticosterone monoacetate was oxidized with chromic trioxide for 5 minutes and converted to 11-dehydrocorticosterone acetate. This compound was chromatographed for 14 to 16 hours in a cyclohexane : benzene : methanol : water (100 : 40 :

TABLE I

Evidence of specificity of the double isotope derivative assay procedure for aldosterone

No. of sample	Source of adrenal vein plasma	H^3/C^{14} ratios after each chromatography			
		1st	2nd	3rd	4th
1	Chronic caval constriction	20.3	1.44	0.47	0.74
2	Chronic caval constriction	54.4	9.48	7.93	8.26
3	Chronic caval constriction	44.7	16.38	15.67	17.20
4	Chronic caval constriction	4.8	0.80	0.52	0.68
5	Normal	14.6	0.54		0.68
6	Chronic caval constriction and cortisone	27.2	7.08	7.22	6.84
7	Chronic caval constriction and cortisone	20.0	2.77	1.90	1.62
8	Chronic caval constriction and cortisone	14.4	1.81	0.68	0.59
9	Chronic caval constriction and cortisone	12.1	0.76	0.46	0.44
10	Chronic caval constriction after hypophysectomy and during ACTH	27.3	10.53	9.46	10.00

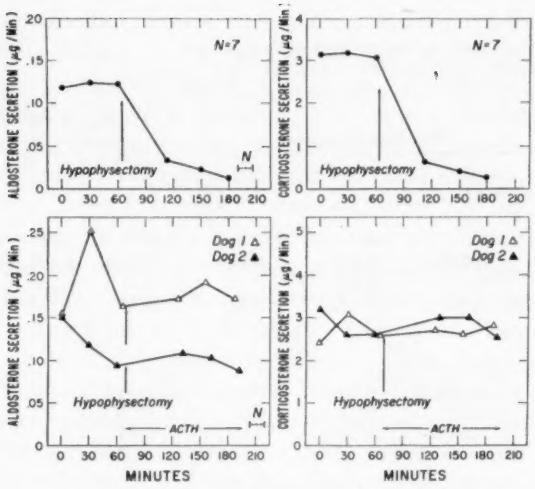

FIG. 1. EFFECTS OF HYPOPHYSECTOMY (UPPER FIGURES) AND THE BLOCKING EFFECTS OF ACTH (0.075 UNIT PER MINUTE) FOLLOWING HYPOPHYSECTOMY (LOWER FIGURES) IN DOGS WITH THORACIC CAVAL CONSTRICTION AND HYPERALDOSTERONISM. The average value of 0.024 μg per minute for aldosterone secretion in 10 normal dogs is represented by the dotted line with the N above it in the lower right corners of the figures on the left.

100:20) system. Because the present data on aldosterone secretion were obtained under different experimental conditions from those reported previously (14), additional evidence on specificity has been provided (Table I). H^3/C^{14} ratios were determined for aldosterone diacetate as described elsewhere (14), after each of 4 chromatographies on adrenal vein plasma from 4 dogs with chronic thoracic caval constriction, one normal dog, 4 of the dogs with chronic thoracic caval constriction given cortisone, and one dog receiving ACTH. For the fourth chromatography, the second chromatographic system employed for routine aldosterone analysis was used and 2-methyl cortisol was added to provide a visible marker. The chromatogram was developed for 18 hours. The H^3/C^{14} ratios were not significantly reduced after the third chromatography with the possible exception of sample 7 (Table I). This finding indicates that no H^3-labeled impurities were present after the third chromatography.

Hypophysectomy was performed by the oral approach. The pituitary was exposed so that it was visible through the dura mater before the initial prehypophysectomy observations were made. After these observations, the pituitary was removed within a 2 to 3 minute period. In Experiment 3, the base of the pituitary stalk was cauterized to destroy any remnant of the pars tuberalis in the first 6 of the 10 dogs; in the remaining 4 dogs, hypophysectomy alone was performed. In Experiment 1, tissue remaining in the sella turcica and the hypothalamus was examined for remnants of adenohypophysis.

RESULTS

Experiment 1. Effects of ACTH following hypophysectomy of dogs with thoracic caval constriction

It has been shown previously (11) that hypophysectomy of dogs with thoracic caval constriction results in a striking fall in the secretion of both aldosterone and corticosterone. In the present experiment, an intravenous infusion of ACTH was given after hypophysectomy of two dogs

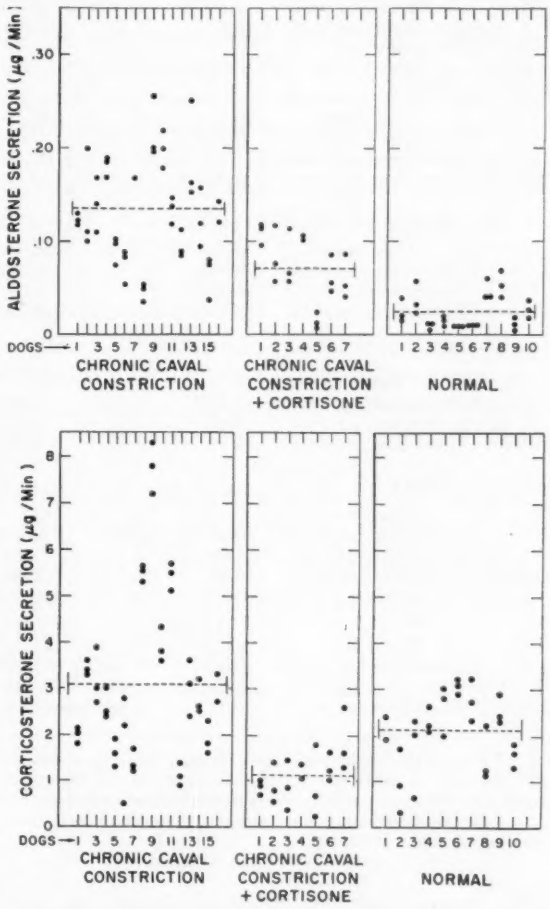

FIG. 2. EFFECT OF CORTISONE (100 MG PER DAY TO DOGS 1 THROUGH 4 AND 200 MG PER DAY TO DOGS 5 THROUGH 7) ON ALDOSTERONE AND CORTICOSTERONE SECRETION IN DOGS WITH CHRONIC THORACIC INFERIOR VENA CAVA CONSTRICTION (CENTER SECTIONS). For comparison, steroid secretion rates in 16 dogs with chronic caval constriction (left sections) and 10 normal dogs (right sections) are presented. The dotted lines show the mean values.

TABLE II

Aldosterone and corticosterone secretion in dogs with secondary hyperaldosteronism treated with cortisone: effects of subsequent hypophysectomy† and ACTH†*

	Initial observations			After hypophysectomy			During ACTH infusion	
	1	2	3	4	5	6	7	8
Dog 1								
Time, *min*	0	26	54	117	150	134	220	227
Aldosterone, *μg/min*	0.097	0.114	0.120	0.058	0.039	0.030	0.254	0.248
Corticosterone, *μg/min*	0.72	1.00	0.90	0.37	0.15	0.13	1.3	1.2
Adrenal blood flow, *ml/min*	3.88	3.88	3.47	2.40	2.18	2.21	2.90	3.18
Dog 2								
Time, *min*	0	27	53	123	163	191	251	
Aldosterone, *μg/min*	0.117	0.057	0.076	0.011	0.011	0.013	0.128	
Corticosterone, *μg/min*	1.4	0.60	0.80	0.09	0.05	0.06	0.90	
Adrenal blood flow, *ml/min*	5.27	4.68	3.80	1.65	1.26	1.09	1.42	
Dog 3								
Time, *min*	0	30	59	136	166	197	260	
Aldosterone, *μg/min*	0.114	0.065	0.056	0.012	0.013	0.008	0.157	
Corticosterone, *μg/min*	1.43	0.84	0.37	0.029	0.021	0.017	1.38	
Adrenal blood flow, *ml/min*	2.92	3.21	2.72	1.92	1.90	1.76	2.58	
Dog 4								
Time, *min*	0		54		162	196	263	271
Aldosterone, *μg/min*	0.104		0.106		0.039	0.043	0.102	0.108
Corticosterone, *μg/min*	1.36		1.07		0.12	0.11	0.94	1.06
Adrenal blood flow, *ml/min*	3.41		1.75		1.02	1.04	1.30	1.38
Dog 5								
Time, *min*	0	31	60					
Aldosterone, *μg/min*	0.012	0.023	0.007					
Corticosterone, *μg/min*	0.67	1.79	0.22					
Adrenal blood flow, *ml/min*	4.06	5.87	5.47					
Dog 6								
Time, *min*	0	29	52	116	144	168		
Aldosterone, *μg/min*	0.047	0.084	0.055	0.004	0.004	0.005		
Corticosterone, *μg/min*	1.00	1.61	1.20	0.05	0.02	0.01		
Adrenal blood flow, *ml/min*	3.09	3.21	2.40	2.34	2.29	2.16		
Dog 7								
Time, *min*	0	0	25					
Aldosterone, *μg/min*	0.052	0.042	0.087					
Corticosterone, *μg/min*	1.63	1.29	2.58					
Adrenal blood flow, *ml/min*	2.31	2.30	2.84					

* Dogs 1 through 4 received 100 mg/day of cortisone intramuscularly (i.m.) for 8 days. Dogs 5 through 7 were given 200 mg/day of cortisone i.m. for 7 days; on the eighth day only 100 mg of cortisone was injected i.m. and 100 mg of cortisone was given orally 3 to 5 hours before collection of adrenal vein blood.

† Hypophysectomy was performed immediately after the third period and the ACTH infusion (0.075 unit/min) was begun after Period 6.

with chronic caval constriction; aldosterone and corticosterone secretion was unaltered. The results are presented in Figure 1 and are compared with the previous finding of decreased steroid secretion following hypophysectomy alone. Histological studies of tissue removed at postmortem examination showed no remaining adenohypophysis in either dog.

Experiment 2. Effects of the chronic daily administration of cortisone on aldosterone and corticosterone secretion in dogs with chronic thoracic caval constriction: subsequent acute effects of hypophysectomy and ACTH

The average value for adrenal vein aldosterone output was 47.3 per cent lower in the seven dogs receiving cortisone (100 to 200 mg per day) than

in untreated dogs with caval constriction ($p < 0.01$, Figure 2). In one of the animals treated with cortisone (Dog 5, Figure 2), all three determinations of aldosterone were at the normal level. The present data are compared with previous results in 16 dogs with chronic caval constriction. Dogs 5 through 7 received 200 mg per day of cortisone and the average value for aldosterone secretion for these animals was 0.045 μg per minute; this value was significantly lower than the average value of 0.135 μg per minute for dogs with caval constriction ($p < 0.02$). The rate of aldosterone secretion for the entire group of seven dogs receiving cortisone was significantly greater than the normal level ($p < 0.01$), whereas aldosterone secretion in the three dogs given 200 mg per day of cortisone was not significantly different from normal ($p > 0.1$).

Corticosterone secretion was 63.4 per cent lower (Figure 2) in dogs receiving cortisone than in dogs with caval constriction alone ($p < 0.01$). Although the response for corticosterone appeared greater than that for aldosterone, the difference was not significant ($p = 0.2$ for the corticosterone : aldosterone ratio). Also, corticosterone output was lower (46.5 per cent) in dogs receiving cortisone than in normal dogs studied under the same experimental conditions ($p < 0.01$). The values for corticosterone secretion in both the untreated dogs with caval constriction and normal dogs are probably high and reflect an increased output of ACTH resulting from the "stress" of adrenal vein cannulation. The rates of adrenal blood flow, inferior vena caval pressures and arterial pressures were essentially the same in the dogs with caval constriction in the absence of or during cortisone administration.

Daily Na balance studies were conducted throughout the period of cortisone administration. Sodium retention continued and Na excretion was less than 5 mEq per day on the day before sacrifice for adrenal vein blood studies in all but one animal (Dog 7); in this animal, Na excretion was 17 mEq per day.

Immediately following the initial observations on the cortisone-treated dogs, hypophysectomy was performed in five of the seven dogs and studies were continued for another two hours. A further marked drop in both aldosterone and corticosterone secretion occurred (Table II). Adrenal blood flow was reduced in four of the five dogs. After the post-hypophysectomy observations, an infusion of ACTH was given to four of the five animals. Adrenal vein aldosterone output increased 8-, 10-, 20- and 2.4-fold with respect to the post-hypophysectomy level in Dogs 1 through 4, respectively, while corticosterone secretion increased 10-, 15-, 81- and 8-fold during the same periods.

Experiment 3. Effects of α- and β-MSH on aldosterone and corticosterone secretion

Synthetic α-MSH (4.75 mg) was assayed in a simple hypophysectomized dog; aldosterone and

TABLE III

Failure of synthetic α-MSH and naturally occurring β-MSH to increase aldosterone and corticosterone secretion in hypophysectomized dogs

	Time	Aldosterone secretion	Corticosterone secretion
	min	*μg/min*	*μg/min*
Control	0	0.010	0.39
	30	0.015	0.09
Infusion of 4.75 mg of synthetic α-MSH	30–60		
After α-MSH	60	<0.005	0.15
	90	<0.005	0.03
Control	0	<0.005	0.01
	30	<0.005	0.01
Infusion of 7 mg of natural β-MSH	30–60		
After β-MSH	60	<0.005	0.02
	90	<0.005	0.01

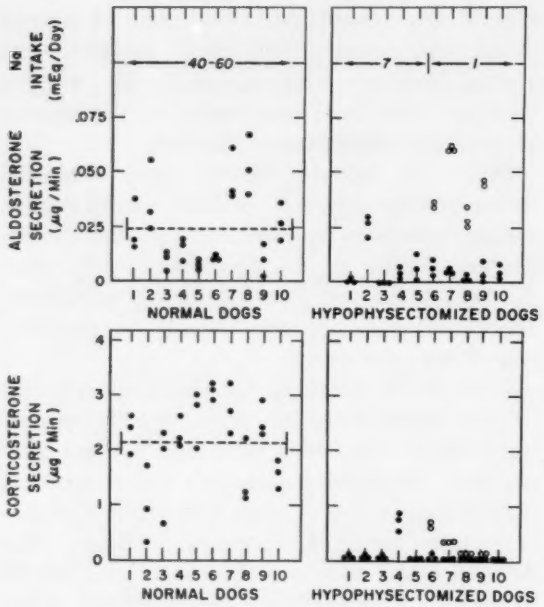

FIG. 3. EFFECTS OF A LOW Na DIET ON ALDOSTERONE AND CORTICOSTERONE SECRETION IN TEN SIMPLE HYPOPHYSECTOMIZED DOGS (SOLID SYMBOLS). The open symbols represent values obtained after 30 to 60 minutes of infusion of ACTH (0.075 unit per minute).

corticosterone secretion was unaffected. Highly purified natural hormone preparations of α- and β-MSH (2.18 mg of α-MSH and 2.0 and 7.0 mg of β-MSH) were without effect on aldosterone and corticosterone secretion in hypophysectomized dogs. The results of the assays of the two largest quantities of MSH are presented in Table III.

Experiment 4. Failure to produce hypersecretion of aldosterone in simple hypophysectomized dogs

A. With a low salt intake. Of the five dogs on a Na intake of 7 mEq per day, only one animal showed a secretion rate similar to the average normal value (Figure 3); all other values were low. A further reduction in Na intake to 1 mEq per day also failed to produce a secretion rate comparable to that for normal dogs ($p < 0.01$; no. = 5). Adrenal blood flow was essentially the same in the present hypophysectomized dogs on a low Na intake as in the normal control animals ($p > 0.2$). Although aldosterone secretion was lower than normal in hypophysectomized dogs on a low salt intake, the secretion rates for some of these dogs were greater than those in two of the three simple hypophysectomized dogs in which aldosterone was undetectable (compare data in Figure 3 and Table IV). The ability of the adrenal cortex to respond to ACTH in hypophysectomized dogs during Na deprivation was demonstrated by giving ACTH after the initial observations were complete (Figure 3). The rate of aldosterone secretion during infusion of ACTH

TABLE IV

Effects of acute thoracic caval constriction in simple hypophysectomized dogs

	Time	Aldosterone secretion	Corticosterone secretion	Adrenal blood flow	Inferior vena caval pressure	Arterial pressure
	min	*μg/min*	*μg/min*	*cc/min*	*mm H_2O*	*mm Hg*
Dog 1						
Control	0	< 0.005	0.036	2.92	62	100
	32	< 0.005	0.056	2.58	55	104
Caval constriction	37					
After constriction	69	< 0.005	0.14	2.02	148	86
	100	< 0.005*	0.15*	2.07*	188*	94*
Dog 2						
Control	0	< 0.005	0.14	3.53	44	121
	27	< 0.005	0.13	2.61	44	131
Caval constriction	36					
After constriction	96	< 0.005	0.29	2.50	155	100
	106	< 0.005	0.25	1.70	155	96
Dog 3						
Control	0	0.008	0.026	2.00	90	100
	27	0.014	0.029	1.98	90	118
Caval constriction	32					
After constriction	57	0.009	0.111	2.81	185	84

* A norepinephrine infusion (100 μg/cc) was given before and during this collection of adrenal vein blood.

increased markedly. The initial rate of corticosterone secretion was very low in all animals and the response in corticosterone output to ACTH was negligible.

B. By acute thoracic inferior vena caval constriction. Two hours after hypophysectomy of three normal dogs, the thoracic inferior vena cava was constricted. The degree of constriction and the resultant changes in venous and arterial pressures were similar to those reported (15) for normal dogs following acute caval constriction. Aldosterone and corticosterone output remained unchanged at the low post-hypophysectomy level (Table IV).

DISCUSSION

Our knowledge of the role of the anterior pituitary in the control of aldosterone secretion and electrolyte metabolism is incomplete. Before aldosterone was discovered, it was recognized that hypophysectomized dogs (16, 17) and rats (18) remain in apparently normal electrolyte balance. The experiments of Lane and de Bodo (16) and of Rolf, Surtshin and White (17) demonstrated Na conservation in hypophysectomized dogs even on a very low Na intake. Also, hypophysectomized dogs with thoracic caval constriction retained Na and formed ascites in the presence of a sufficiently elevated venous pressure (1). The finding of Farrell, Rauschkolb and Royce (3) of only a moderately reduced rate of aldosterone secretion following hypophysectomy in the dog was in agreement with earlier studies on electrolyte metabolism.

Other workers, however, have reported a striking fall in aldosterone output following hypophysectomy. In rats, Singer and Stack-Dunne (19) reported an 83 per cent reduction in aldosterone secretion after hypophysectomy. Ganong and associates (20) also found an 83 per cent lower value for aldosterone production in simple hypophysectomized dogs than in normal animals. In experimental secondary hyperaldosteronism in the dog, hypophysectomy resulted in an average reduction of 90 per cent in aldosterone and corticosterone secretion (11). The fall in aldosterone secretion following hypophysectomy of normal anesthetized animals may be exaggerated because of the initial high output which results from the stress of adrenal vein cannulation. However, in unanesthetized dogs with chronic thoracic caval constriction, aldosterone secretion occurs at the same high rate as in anesthetized dogs with caval constriction which are stressed by adrenal vein cannulation (personal observations). The striking drop in aldosterone secretion following hypophysectomy of dogs with thoracic caval constriction emphasizes the important role of the anterior pituitary in the production of aldosterone. Maintenance of a normal electrolyte balance in hypophysectomized dogs in the presence of a low aldosterone output may be the result of the markedly reduced rate of glomerular filtration (21, 22).

In contrast to the situation in the dog, man appears to be fragile. There are several well documented cases of hypopituitarism with salt loss and the resultant clinical features of adrenocortical insufficiency (23). Hernando and associates (7) reported studies of several hypophysectomized patients or patients with hypopituitarism with urinary aldosterone outputs at the lower limit of normal. In one of these patients, a low Na intake failed to increase urinary aldosterone excretion on two occasions. The experiments of Llaurado (24) have demonstrated that the increase in urinary aldosterone excretion and the reduction in the urinary Na/K ratio were consistently greater after nonendocrinological operations than following total hypophysectomy. These data indicate that the response in aldosterone production to surgery is less following hypophysectomy, a finding which suggests the necessity of the anterior pituitary for a normal response in aldosterone secretion to surgery.

What anterior pituitary hormones are important in the control of aldosterone secretion? The response to different corticotropin preparations (3, 7, 8–10) suggests that ACTH influences aldosterone production. The present data show that the 90 per cent drop in aldosterone and corticosterone secretion which follows hypophysectomy was blocked completely by an ACTH preparation. Since the possibility of a contaminating substance in corticotropin preparations cannot be excluded, the effect of inhibition of ACTH secretion on adrenal steroid production was studied.

In dogs with secondary hyperaldosteronism treated with cortisone, a decrease in both aldosterone and corticosterone secretions occurred in the absence of a detectable change in cardiovascular function. The ACTH content of the adenohypophysis has been demonstrated to be markedly decreased during treatment of normal dogs with similar doses of cortisone and cortisol (25). It appears likely, therefore, that the drop in aldosterone and corticosterone production was secondary to decreased output of ACTH. The data provide more conclusive evidence than those from administration of corticotropin preparations that ACTH plays an important role in aldosterone production. Also, following hypophysectomy and a resultant fall in aldosterone secretion in these ACTH-suppressed dogs, administration of ACTH restored aldosterone and corticosterone output to the very high levels characteristic of dogs with caval constriction. In earlier chronic studies (2), failure of ACTH to prevent the drop in urinary aldosterone excretion following hypophysectomy of dogs with caval constriction seems explicable on the basis of an inadequate level of venous pressure; reconstriction of the thoracic inferior vena cava in these dogs and a resultant further rise in venous pressure was followed by hyperaldosteronuria.

The values for corticosterone secretion in dogs with caval constriction and in normal dogs (Figure 2) are markedly elevated in comparison with the basal rate of corticosterone secretion in trained unanesthetized dogs (personal observations). These high rates of corticosterone secretion reflect the "stress" secondary to adrenal vein cannulation. Hilton and associates (26) reported near maximal output of cortisol in anesthetized dogs during adrenal vein cannulation. Also of interest is the finding of higher values for corticosterone secretion in normal dogs than in the cortisone-treated dogs with caval constriction, while the reverse situation was observed for aldosterone output. These data suggest that the stimulus which increased corticosterone secretion (presumably increased ACTH output) in normal dogs did not similarly influence aldosterone production. The present observations favor the view that ACTH provides important support for steroidogenesis by the adrenal cortex and does not necessarily play an initiative regulatory role in the control of aldosterone secretion. This view is in agreement with the finding of Liddle, Duncan and Bartter (8) that patients with secondary hyperaldosteronism excrete normal amounts of 17-hydroxycorticoids.

In the experiments of Farrell, Banks and Koletsky (25), cortisone and cortisol, 100 mg per day, markedly depressed the ACTH content of the anterior pituitary in normal dogs, and the rates of secretion of cortisol and corticosterone were very low. In contrast, aldosterone secretion was essentially the same in their steroid-treated dogs as in their normal control animals. Their dogs were bled from the adrenolumbar vein over a four hour period. Since bleeding stimulates aldosterone secretion (27, 28), it is possible that a fall in aldosterone output was obscured by the effect of bleeding. In the present experiments only 20 to 30 cc of blood was removed for each group of analyses and this was replaced immediately.

The further decrease in aldosterone secretion following hypophysectomy of the cortisone-treated, ACTH-suppressed dogs of the present study raises the question of the influence of anterior pituitary hormones other than ACTH on aldosterone production. There is, however, the possibility that ACTH secretion was not completely suppressed by cortisone. The present α- and β-MSH preparations had no effect on aldosterone and corticosterone secretion. During chronic balance studies, thyrotropin and growth hormone preparations resulted in marked Na retention in hypophysectomized dogs with thoracic caval constriction, and discontinuation of both hormones was followed by increased Na excretion (1). It was suggested (1) that the effects of both thyrotropin and growth hormone were at least partially indirect and mediated through an increase in venous pressure which occurred concurrently with the Na retention. Canter and coworkers (29) have recently reported that thyroidectomy resulted in a Na diuresis in dogs with thoracic caval constriction and ascites. Conclusive evidence for a direct effect of thyrotropin and growth hormone is lacking and there is evidence to the contrary for growth hormone (5). Finally, it should be pointed out that another highly active ACTH-like anterior pituitary hormone with potent effects on aldosterone secretion is a possibility.

The marked effects of hypophysectomy and the importance of ACTH in aldosterone production raise the question of the capacity of the adrenal cortex to secrete increased amounts of aldosterone in the absence of adequate anterior pituitary function. The secretion rates for aldosterone in the present chronic simple hypophysectomized dogs on a low Na intake were below the normal level. The duration of the low Na intake was greater than that required for a response in normals to altered electrolyte intake (8, 30, 31). Several of these low values for aldosterone secretion in hypophysectomized dogs on a low Na intake were slightly higher, however, than aldosterone secretion in the two of the three dogs which were on a normal Na intake and aldosterone output was measured two hours after hypophysectomy (Table IV). This finding may indicate some adrenocortical stimulation by the low Na diet in the simple hypophysectomized dogs. Corticosterone output was extremely low and apparently unaffected by the low Na diet. These observations are in agreement with the earlier reports of Singer and Stack-Dunne (19) and Eisenstein and Hartroft (32) that a low Na diet increased aldosterone production in normal rats while corticosterone output was unaltered or declined. Also, Rosnagle and Farrell (33) found no influence of a low Na diet on cortisol output in normal dogs whereas aldosterone secretion was doubled.

Following acute thoracic caval constriction of simple hypophysectomized dogs, aldosterone secretion failed to increase with essentially the same alterations in cardiovascular function as those produced previously in normal dogs (15). In normal dogs, caval constriction consistently increased aldosterone secretion (15). The data agree with the finding (11) that the acute effect of hypophysectomy in dogs with hyperaldosteronism is a marked fall in aldosterone secretion. In previous chronic experiments (2) it was found, however, that increased aldosterone output in urine and presumably an elevated level of circulating aldosterone occurred frequently in association with marked Na retention and ascites in hypophysectomized dogs with caval constriction. More recently, chronic studies of conscious hypophysectomized dogs with caval constriction (personal observations) have demonstrated that aldosterone secretion, although markedly reduced, remains sufficiently elevated to explain the associated hyperaldosteronuria and Na retention. These data, then, provide evidence of hypersecretion of aldosterone in the absence of the anterior pituitary and, thereby suggest an extra-pituitary origin for the aldosterone stimulating hormone, demonstrated previously by cross circulation experiments (14).

The present results are consistent with certain studies and proposed schema for the biogenesis of aldosterone *in vitro* from glomerulosa slices (34). Ayres and associates (34) have proposed that corticosterone is the immediate precursor to aldosterone in the major pathway of aldosterone synthesis; under certain experimental circumstances 50 to 92 per cent of aldosterone produced *in vitro* was derived from corticosterone. In the present study, there was a striking similarity in the changes in aldosterone secretion and corticosterone secretion following hypophysectomy and during ACTH administration to hypophysectomized dogs with caval constriction. During cortisone suppression of ACTH secretion, both aldosterone and corticosterone production fell in dogs with caval constriction. During ACTH administration to hypophysectomized dogs on a low Na intake, corticosterone output failed to increase while aldosterone production reached the upper limit of the normal range. These results may reflect inadequate substrate (precursors) for corticosterone production due to the chronic effects of hypophysectomy and atrophy of the zona fasciculata and reticularis. Sufficient corticosterone (if it is the precursor for aldosterone) was present for conversion to aldosterone and this presumably occurred in the zona glomerulosa. Although corticosterone secretion was markedly reduced in the present hypophysectomized dogs on a low Na diet the concentration of corticosterone in adrenal vein plasma was several times higher than that of aldosterone so that increased aldosterone production by ACTH might have occurred by conversion of corticosterone to aldosterone.

SUMMARY AND CONCLUSIONS

An ACTH preparation given following hypophysectomy of two dogs with thoracic caval con-

striction and hyperaldosteronism prevented the usual fall in aldosterone and corticosterone secretion which follows anterior pituitary ablation. To inhibit ACTH secretion, 100 to 200 mg per day of cortisone was given for eight days to seven dogs with experimental secondary hyperaldosteronism. Aldosterone and corticosterone production was significantly lower than in dogs with chronic ascites without cortisone therapy, and in one of the animals aldosterone output was depressed to normal. Subsequent hypophysectomy of five of these seven ACTH-suppressed dogs resulted in a further decline in aldosterone and corticosterone output. In four of these five animals subjected to hypophysectomy, the ACTH preparation increased aldosterone and corticosterone secretion to or above the control levels. In simple hypophysectomized dogs, α- and β-MSH were without effect on aldosterone and corticosterone secretion. Attempts to produce hypersecretion in simple hypophysectomized dogs failed: *1*) adrenal vein aldosterone output was significantly lower than normal in ten simple hypophysectomized dogs during maintenance on a low Na diet; *2*) in three simple hypophysectomized dogs, acute thoracic caval constriction failed to produce an increase in aldosterone secretion. Although aldosterone output was lower in the hypophysectomized dogs on a low Na intake than in normal dogs on normal electrolyte intake, the data suggest some adrenocortical stimulation in the absence of the anterior pituitary. The response in aldosterone secretion to the ACTH preparation in simple hypophysectomized dogs on a low Na diet was striking, whereas the increase in corticosterone production was slight. It is concluded that the anterior pituitary plays an important role in the increased production of aldosterone in experimental secondary hyperaldosteronism. It seems likely that ACTH supports steroidogenesis at a very high level.

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EFFECT OF INTRAVENOUS INFUSIONS OF ETHYL ALCOHOL ON ESTIMATED HEPATIC BLOOD FLOW IN MAN *

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The effect of ethyl alcohol on the splanchnic or liver blood flow has been a matter of debate. According to earlier investigations [Starling (1)] ethanol has little effect on the cardiovascular system, its influence being mainly confined to a dilatation of the vessels of the skin and of the mucous membranes. The investigation of Smythe, Heinemann and Bradley on dogs (2) supported this concept. However, Mendeloff (3) in studies on man observed a striking increase in the splanchnic blood flow during intravenous infusions of ethyl alcohol.

In this laboratory, metabolism of ethanol is now being studied. During these studies no effect of ethanol on the splanchnic blood flow could be found. The aim of this paper is to present these data.

MATERIAL AND METHODS

Six subjects were studied. Each was a healthy volunteer with no signs of cardiovascular or liver disease. In two, the procedure was repeated after several weeks had elapsed.

The technique was essentially that described by Bradley, Ingelfinger, Bradley and Curry (4). All studies were performed in the morning after an overnight fast. The subjects were recumbent. The sulfobromophthalein (BSP) solution was infused through a polythene catheter inserted a short distance into one of the antecubital veins; peripheral blood was obtained from a short polythene catheter in a brachial artery. The right hepatic vein was catheterized percutaneously through the femoral vein. The catheter used has been described by Ödman (5) as a modification of that of Gidlund (6).

The experiments were started with an estimation of the plasma volume. For this purpose 100 mg of BSP was given as a single injection and blood was sampled after 3, 5, 7 and 9 minutes from the artery for determination of the BSP space. An intravenous priming dose of 150 mg BSP was then given, followed by the sustaining infusion of BSP. This infusion was administered at a constant rate with a motor-driven syringe (7) so that approximately 2 ml of fluid was injected per minute. The bottle containing the infusion fluid was placed on an automatic balance enabling continuous control of the infused amount of fluid. As a rule approximately 3 mg BSP per minute per square meter body surface area was given. Sampling for analysis was not begun until after 30 minutes of infusion, to allow for equilibration between amount of infused and removed dye. Samples from the hepatic vein and the brachial artery were withdrawn simultaneously at 5 to 10 minute intervals. The basal splanchnic blood flow was determined over two periods of 5 to 10 minutes.

In 5 experiments a priming dose of 10 g ethanol [1] in 100 ml physiological saline was given intravenously during 5 minutes before the start of the sustaining infusion. A sustaining infusion ranging between 0.18 and 0.50 g of alcohol per minute was delivered in 3 to 5 ml saline. The ethanol infusion was continued, on the average, for 35 minutes. During the alcohol infusion, samples from the hepatic vein and the brachial artery were simultaneously drawn at 5 to 10 minute intervals. After finishing the ethanol infusion further sampling was made for 3 more determinations of the splanchnic blood flow.

The subjects did not show any sign of alcohol intoxication during the studies. There was no effect on pulse rate and blood pressure during the alcohol infusion except a slight tachycardia when the priming dose of ethanol was given.

Methods of analysis. Before sampling, the salt solution in the venous catheter was washed out by withdrawing 4 ml of blood, which was discarded. Samples were then obtained in heparinized tubes for analysis. The BSP determinations were made in duplicate in the Beckman DU spectrophotometer by using the principle of the second method described by Gaebler (8). The hematocrit was determined after spinning the blood in the Wifug hematocrit centrifuge at a rate of 3,000 rpm for 30 minutes. The estimated splanchnic blood flow (ESBF) and the BSP extraction ratio were calculated

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[1] The ethanol used was a redistilled product made from cellulose and distributed as a 96 per cent solution. It was manufactured by a Swedish state factory. The only contaminants are: methanol, less than 0.5 g per L; and acetaldehyde, less than 0.005 g per L.

TABLE I

Sulfobromophthalein (BSP) data and ethanol data in eight ethanol experiments

Case no.	Sex	Age	BSA	Time after start of infusion	Infused amount BSP	Arterial plasma BSP conc.	Percentage BSP extraction	BSP clearance	ESBF	Mean value	Infused amount ethyl alcohol	Arterial alcohol conc.	Hematocrit*
			m²		*mg/min*	*mg/100 ml*		*ml/min*	*ml/min*		*g/min*	*g/L*	%
53/59	M	39	2.07	Before	10.05	2.20†	65				0.177		41.0
				↓		2.21	60	450	1,220	1,200			
						2.32	59	410	1,170				
		Priming 10 g		0.0							Inf. in		
				4.0		2.38	58	410	1,200		↓		
				13.5		2.65	55	360	1,070				
				23.0		2.89	48	330	1,090				
				33.0		3.33	50	270	940	1,020			
				34.0	10.42						out		
				42.0		3.62	47	270	940				
				52.0	10.57	3.98	39	240	950				
				62.0		4.33	37	220	990				
54/65	M	40	2.00	Before	7.11	2.16	51				0.177		39.0
				↓		1.96	45	380	1,280	1,320			
						1.92	45	370	1,360				
		Priming 10 g		0.0							Inf. in		
				5.5		1.96	52	360	1,200		↓		
				15.5		1.91	52	380	1,190				
				26.5		1.87	50	380	1,230				
				36.0		1.81	52	400	1,280	1,240			
				37.0							out		
				46.0		1.75	51	410	1,300				
				56.0		1.73	54	410	1,280				
				66.0		1.77	53	400	1,220				
57	M	31	1.93	Before	9.28	2.67	29				0.201		35.0
				↓		2.76	32	320	1,630	1,610		0.000	
						2.86	26	300	1,640			0.000	
				0.0							Inf. in		
				4.5		3.10	26	270	1,620		↓	0.046	
				15.0		3.17	27	290	1,650			0.067	
				25.5		3.49	24	240	1,460			0.077	
				35.5		3.77	27	230	1,380	1,420		0.095	
				36.0							out		
				46.0		4.03	28	210	1,210			0.044	
				56.5		4.30	19	200	1,320			0.021	
				66.0		4.69	25	190	1,360			0.013	

* All the subjects were blood donors; this may explain the slightly subnormal hematocrit values.

† In Experiment 53/59 the arterial BSP plasma concentration rose with time. This rise does not significantly influence the calculated blood flow values (9, 10).

TABLE I—(*Continued*)

Case no.	Sex	Age	BSA	Time after start of infusion	Infused amount BSP	Arterial plasma BSP conc.	Percentage BSP extraction	BSP clearance	ESBF	Mean value	Infused amount ethyl alcohol	Arterial alcohol conc.	Hematocrit*
			m²		*mg/min*	*mg/100 ml*		*ml/min*	*ml/min*		*g/min*	*g/L*	%
58	M	21	1.86	Before	6.10	1.72	47	310	1,170	1,160	0.175		40.5
				↓		1.84	43	300	1,150			0.000	
						1.93	44					0.003	
				0.0				290	1,100		Inf. in		
				4.0		2.02	44	270	1,060		↓	0.061	
				14.0		2.18	41	250	1,090			0.078	
				24.0		2.37	35	240	1,140	1,090		0.083	
				34.0		2.46	37					0.112	
				35.0				240	1,200		out		
				44.0		2.52	31	220	1,110			0.076	
				54.5		2.70	34	200	960			0.020	
				64.5	↓	2.89	36					0.015	
59/53	M	39	2.07	Before	6.37	1.29	49	480	1,570		0.181		39
				↓		1.31	52	460	1,550	1,560		0.000	
						1.35	45					0.000	
		Priming 10 g		0.0				480	1,700		Inf. in		
				4.0		1.34	47	460	1,720		↓	0.410	
				14.5		1.38	41	470	1,890			0.250	
				25.0		1.35	41					0.240	
				36.0				450	1,670	1,650	out		
				37.5		1.42	46	440	1,560			0.210	
				46.0		1.44	47	420	1,520			0.180	
				56.0		1.49	45	400	1,480			0.150	
				66.5	↓	1.58	42					0.120	
60	M	24	1.98	Before	6.55	2.12	51	340	1,160				40.0
				↓		2.04	47	320	1,140	1,150		0.030	
						2.05	46					0.020	
		Priming 10 g		0.0							Inf. in		
				5.0		1.99	43	340	1,260		0.249		
				15.0		1.99	49	330	1,190		0.426	0.650	
				25.0		1.90	45	350	1,260		0.456	0.570	
				35.5		1.91	42	340	1,310	1,260	0.492	0.530	
				36.0								0.600	
				45.5		2.04	42	310	1,230		out		
				55.5		2.14	38	300	1,250			0.450	
				65.5	↓	2.25	33	280	1,330			0.400	
												0.400	

TABLE I—(*Continued*)

Case no.	Sex	Age	BSA	Time after start of infusion	Infused amount BSP	Arterial plasma BSP conc.	Percentage BSP extraction	BSP clearance	ESBF	Mean value	Infused amount ethyl alcohol	Arterial alcohol conc.	Hematocrit*
			m²		*mg/min*	*mg/100 ml*		*ml/min*	*ml/min*		*g/min*	*g/L*	%
61	M	27	2.07	Before	6.15	0.81	79				0.430		42.5
				↓	↓	0.75	75	840	1,890			0.000	
						0.76	78	810	1,840	1,870		0.000	
		Priming 10 g		0.0							Inf. in		
				7.0		0.82	72	750	1,720		↓	0.650	
				17.0		0.86	72	720	1,700			0.500	
				27.5		0.90	71	690	1,660			0.620	
				38.0		1.00	80	620	1,410	1,430		0.590	
				39.0							out		
				48.5		1.16	74	530	1,190			0.440	
				59.0		1.21	79	510	1,150			0.460	
				69.0	↓	1.13	76	550	1,220			0.460	
65/54	M	40	2.00	Before	7.34	1.82†	54				0.473		37.5
				↓	2.72	1.77	50	430	1,320			0.000	
					6.53	1.18	48	460	1,490	1,410		0.000	
				0.0							Inf. in		
				3.0	8.07	1.50	59	340	1,010		↓	0.110	
				13.0		1.71	55	450	1,280			0.300	
				22.5		1.77	52	450	1,350			0.350	
				31.5		1.88	51	420	1,300			0.450	
				32.0						1,300			
				41.0		1.84	48	440	1,440		out		
				50.5		1.94	50	410	1,330			0.240	
				60.0	↓	2.01‡	50	390	1,260			0.170	
												0.220	

‡ Hemolysis.

from the following formulae:

$$\text{ESBF} = \frac{\text{removal} \times 100}{(p - h)\,(1 - \text{Hct})};$$

$$\text{removal} = I \pm \frac{\Delta p \cdot \text{BSP space}}{100};$$

$$\text{BSP clearance} = \frac{\text{removal} \times 100}{p};$$

$$\text{extraction ratio} = \frac{(p - h)\,100}{p};$$

where I = infusion rate (milligrams per minute), p = arterial = peripheral plasma concentration (milligrams per 100 ml plasma), h = liver vein plasma concentration (milligrams per 100 ml plasma), Δp = change in p in milligrams per 100 ml plasma per minute, and Hct = hematocrit value in per cent.

Alcohol determination was made according to Bonnichsen and Lundgren (9).

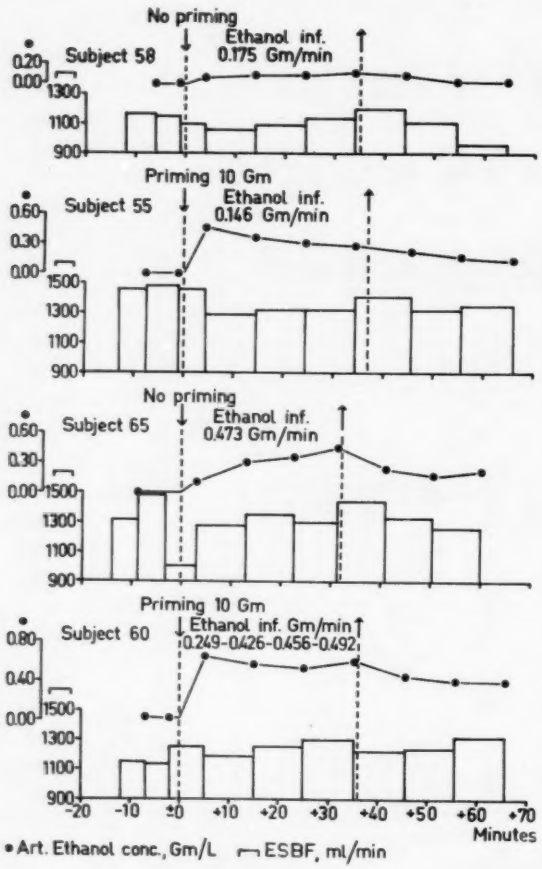

FIG. 1. THE ESTIMATED SPLANCHNIC BLOOD FLOW IN MILLILITERS PER MINUTE AND THE ETHANOL BLOOD CONCENTRATION IN GRAMS PER LITER IN FOUR OF EIGHT EXPERIMENTS.

RESULTS

The data from the eight studies are given in Table I. In Experiments 57, 58 and 65 no priming doses of ethanol were given, resulting in a slow increase in the blood concentration of ethanol. The infused amount of ethanol ranged between 0.18 and 0.50 g per minute. The arterial ethanol concentration in Experiments 53 and 54 was, for technical reasons, not obtained. In the remaining experiments the maximal concentration ranged between 0.10 and 0.65 g ethanol per L blood.

No effect of ethanol on the estimated splanchnic blood flow was observed in any of the subjects studied. Further, ethanol did not influence the BSP clearance. In Figure 1 four studies are illustrated.

DISCUSSION

The difference between our results and those of Mendeloff are difficult to explain. Essentially the same technique has been used in the two investigations. In Mendeloff's five experiments the blood concentration of ethanol ranged between 0.14 and 0.82 g ethanol per L, levels corresponding to our values of 0.10 to 0.65 g ethanol per L. However, our subjects were healthy volunteers while Mendeloff studied patients with gastric ulcer. Also, we gave no sedation while Mendeloff gave a subcutaneous injection of 60 mg phenobarbital sodium before the study. Such sedation would probably tend to depress the effect of ethanol on the splanchnic blood flow.

To find out if there is a different response to very low peripheral alcohol concentrations we kept the blood ethanol below 0.15 g per L in two experiments. The results in these experiments were the same as in those with blood alcohol concentrations above 0.15 g per L. There was also no effect on the ESBF in those experiments in which the blood concentration was raised from zero up to 0.65 g per L within five minutes (priming experiments).

In three experiments (nos. 53, 57 and 61) there was slight decrease in ESBF with time. As the same tendency was found in another study (11) without any drug, we suggest that this fall was a spontaneous change not due to ethanol.

SUMMARY

The effect of ethanol on the splanchnic blood flow was studied in eight experiments on six healthy volunteers using the sulfobromophthalein (BSP) method. No sedation was used. The peripheral ethanol concentration ranged between 0.10 and 0.65 g per L. There was no significant effect of the ethanol in any of the eight studies.

ACKNOWLEDGMENT

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NONDIALYZABLE HEXOSE OF HUMAN SYNOVIAL FLUID*†

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Studies that show differences in the components of synovial fluids in osteoarthritis and rheumatoid arthritis may be helpful diagnostically. Synovial fluids from joints involved by rheumatoid arthritis contain a high concentration of protein, and electrophoretic studies reveal increased α_2-globulins (1, 2). Since this protein fraction contains the highest concentration of hexose among the electrophoretically separated proteins of serum (3), determination of nondialyzable hexose concentration in synovial fluid was undertaken. In synovial fluid from osteoarthritic joints the nondialyzable hexose concentration, determined with the anthrone reagent, was similar to or lower than the normal level, while in synovial fluid from joints involved by rheumatoid arthritis it was elevated.

The first step in this study was to identify the carbohydrate components of dialyzed synovial fluid that contributed to the anthrone reaction. Synovial fluid was dialyzed and then lyophilized. The lyophilized solids were hydrolyzed in sulfuric acid, and the carbohydrates in the hydrolysate were separated by a combination of zone electrophoresis in borate buffer and paper chromatography. Galactose, mannose, small amounts of fucose and glucose, and hyalobiuronic acid were found.

MATERIALS AND METHODS

I. Identification of carbohydrate components

Synovial fluid from normal subjects. Synovial fluid was aspirated from apparently normal knee joints of deceased subjects. Fluids from 8 to 10 normal knees were pooled to provide about 10 ml. Three different pools of normal synovial fluid were collected. The pooled fluids were diluted with an equal volume of a buffer (0.03 M $NaHCO_3$ and 0.15 M NaCl, pH 8.1), dialyzed against 500 ml of distilled water (changed twice daily) for 48 hours at 2° C, and lyophilized.

Synovial fluid from patients with primary osteoarthritis or rheumatoid arthritis.[1] About 10 ml of synovial fluid was obtained from the involved knee joint of 1 patient with primary osteoarthritis and from 2 patients with rheumatoid arthritis. These fluids were treated in the same manner as the pooled normal synovial fluids.

Normal serum. The total protein-bound hexose of pooled normal serum was precipitated with ethanol according to the method of Winzler (4), and dried in a vacuum over P_2O_5.

Hydrolysis. Lyophilized solids (80 mg) of pooled normal synovial fluid were hydrolyzed in 1 ml of 2 N H_2SO_4 in a sealed tube for 2 hours at 100° C. The hydrolysate was adjusted to pH 5.0 with $Ba(OH)_2$, and the insoluble $BaSO_4$ removed by centrifugation. Lyophilized solids of osteoarthritic synovial fluid (100 mg), rheumatoid synovial fluid (100 mg), and the dried precipitate of normal serum (100 mg) were similarly hydrolyzed and neutralized.

Zone electrophoresis. The technique employed was modified from that of Müller-Eberhard and Kunkel (5). Blocks ($4 \times 13 \times \frac{1}{4}$ inches) of polyvinyl chloride (Geon Resin no. 435, obtained from B. F. Goodrich) were used. The hydrolysate of normal synovial fluid was applied to the block in a slit ($\frac{1}{2} \times \frac{1}{8} \times \frac{1}{4}$ inches) 3 inches from the cathodal end of the block. A mixture of authentic glucose, galactose and mannose was applied to a slit in the block 2 inches lateral to the hydrolysate. Electrophoresis was carried out for 9 hours in borate buffer (0.05 M $Na_2B_4O_7 \cdot 10\ H_2O$ and 0.15 M H_3BO_3, pH 8.5) at a voltage gradient of 12.5 volts per cm at 2° C. The block was blotted dry and divided longitudinally into two equal parts. Each part was cut into ¼ inch segments starting at the anodal end. Each segment was placed in a 15 ml sintered glass funnel (medium porosity), and elution was performed with 3 additions (2.5 ml each) of distilled water by applying air pressure to the funnel. The final volume of each eluate was made up to 8 ml. After analysis for hexose, the eluates of some segments

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§ Markle Scholar in Medical Science.

[1] For simplicity, synovial fluids obtained from involved joints of patients with osteoarthritis or rheumatoid arthritis will be termed osteoarthritic synovial fluid and rheumatoid synovial fluid, respectively.

were pooled to obtain a larger quantity of the isolated carbohydrates.

Electrophoresis of the hydrolysates of osteoarthritic synovial fluid, rheumatoid synovial fluid, and normal serum was similarly performed.

Removal of sodium borate. It was necessary to remove sodium borate from the eluates prior to chromatography. The eluates containing *hexoses* were passed through a column (5 × 0.9 cm) of Amberlite IR-120 (H^+ form). The effluent was evaporated to dryness in a vacuum, methanol (3 additions of 5 ml each) added, and the methyl borate removed by vacuum distillation at 30° C (6). The eluates containing *hyalobiuronic acid* were passed through a column (3 × 0.9 cm) of equal parts (by weight) of carbon and celite (7). The sodium borate was washed from the column with distilled water (3 additions of 10 ml each). The hyalobiuronic acid was then eluted from the column with 10 ml of 30 per cent (vol/vol) aqueous ethanol.

Chromatography. Ascending paper chromatography was performed using Whatman no. 1 paper at room temperature for 16 hours. Hexoses were separated in isopropyl alcohol-water (8:2) (8), and the chromatograms were stained with aniline hydrogen phthalate. Eluates containing hyalobiuronic acid were chromatographed in butanol-acetic acid-water (6:2:2), and the chromatograms were stained with either aniline hydrogen phthalate or *p*-dimethylaminobenzaldehyde in HCl (8).

Analytical methods. All the eluates were analyzed for hexoses by an anthrone method (9). Anthrone reagent was prepared by adding 100 ml of C.P. H_2SO_4 to 0.2 g of anthrone. Five ml of anthrone reagent was added to Pyrex test tubes (25 × 150 mm) in water at 10° C. An aliquot (2.5 ml) of each eluate was layered on top of the anthrone reagent. A reagent blank (2.5 ml of the $NaHCO_3$-NaCl buffer) and the hexose standard (1 ml containing 50 μg per ml of galactose and 50 μg per ml of mannose plus 1.5 ml of buffer) were similarly prepared. The tubes were covered with glass marbles, shaken, removed from the water, and allowed to stand at room temperature for 5 minutes. The tubes were immersed in water at 100° C for exactly 6 minutes, and then cooled in water at 10° C for 5 minutes. Optical density was determined in a Beckman DU spectrophotometer at 625 mμ.

Some of the eluates were also analyzed for hexosamine (10) and hexuronic acid (11). Lyophilized nondialyzable solids of synovial fluids were analyzed for hexose, hexosamine and nitrogen (12).

II. Determination of nondialyzable hexose concentration

Synovial fluid was obtained from two groups of subjects with apparently normal knee joints. The first group consisted of 22 deceased subjects of varying ages who had no evidence of acute illness. The second group, was composed of living subjects in coma from an acute cerebral vascular accident. The methods of aspiration and preservation of the fluid are described elsewhere (10). Synovial fluid was also obtained from *involved* knee joints of patients with active rheumatoid arthritis or primary osteoarthritis. The diagnosis of definite rheumatoid arthritis fulfilled the criteria of the American Rheumatism Association (13). The diagnosis of primary osteoarthritis was established by history, physical examination, X-ray changes, normal erythrocyte sedimentation rate, and negative latex fixation test. Serum was obtained from the living subjects at the time synovial fluid was aspirated.

Dialysis. To a weighed aliquot (0.2 g) of normal or osteoarthritic synovial fluid was added 2.5 ml of a buffer (0.03 M $NaHCO_3$, 0.15 M NaCl, pH 8.1). To rheumatoid synovial fluid (0.15 g) 3 ml of buffer was added. Each diluted fluid was mixed, added to cellulose casing (Visking, $\frac{8}{32}$ inch) and dialyzed against 500 ml of buffer changed twice daily for two days at 2° C. To weighed aliquots (0.25 g) of serum from normal subjects or patients with osteoarthritis was added 3 ml of buffer. A smaller aliquot (0.15 g) of serum from patients with rheumatoid arthritis was similarly diluted with buffer. The diluted sera were then dialyzed against buffer.

Alcohol precipitation. In a few synovial fluids and sera, the values for nondialyzable hexose were compared with protein-bound hexose precipitated by alcohol. Weighed aliquots similar to those described above were taken. Normal synovial fluid was diluted 1:5 with tap water; rheumatoid synovial fluid was diluted 1:1; serum was not diluted. To the diluted fluids and sera 10 ml of cold absolute ethanol was added with constant stirring. After standing for 30 minutes at 2° C, the tubes were centrifuged, the precipitate washed once in ethanol, and then dissolved in 2 or 3 ml of buffer.

Hexose analysis. To 5 ml of anthrone reagent in Pyrex test tubes immersed in water (10° C), 0.5 ml buffer and 2 ml [2] of diluted dialyzed synovial fluid were carefully added down the side of the tube. For analysis of serum, 1.5 ml of buffer and 1 ml of diluted, dialyzed serum were used. A hexose standard (1 ml containing 50 μg per ml of galactose and 50 μg per ml of mannose plus 1.5 ml of buffer) and a reagent blank (2.5 ml of buffer) were similarly prepared. The determination was carried out as described above.

Protein determination. A biuret method was used (14). When the total volume of synovial fluid was less than 0.3 g a modified Folin method (15) was employed to permit analysis of protein in 0.05 g of synovial fluid. Normal serum, whose nitrogen was determined by a micro-Kjeldahl method (12), was used as a standard. Values for protein obtained by the biuret and Folin method on the same synovial fluids agreed within 5 per cent.

Anthrone reaction of isolated hyaluronidase-digested hyaluronate. Synovial fluid was digested with hyaluronidase, and the hyaluronate fragments isolated by zone electrophoresis in a phosphate buffer (0.05 M, pH 6.5) on blocks of polyvinyl chloride (16). The molar ratio

[2] The density of diluted synovial fluid is 1, and grams and milliliters may be used interchangeably.

TABLE I

Analyses of nondialyzable solids of human synovial fluid

	Weight of solids (mg/g) of synovial fluid	Hexose	Hexosamine	Nitrogen
		%	%	%
Normal	19.9	1.8	5.1	12.2
Normal	17.7	1.8	5.5	
Normal	17.0	2.0		
Osteoarthritis	39.8	1.6	3.1	12.1
Rheumatoid arthritis	64.4	1.3	2.0	10.2
Rheumatoid arthritis	43.8	1.5	2.8	

of hexuronic acid to hexosamine of the isolated hyaluronate fragments was 1. No protein was detected by the Folin method (15) and chromatography after acid hydrolysis revealed hexosamine but no amino acids. The anthrone reaction of an aliquot of this material containing 400 μg of hexuronic acid per ml was determined.

RESULTS

I. Identification of carbohydrate components

Analytical data. The hexose, hexosamine and nitrogen content of the nondialyzable solids of normal, osteoarthritic and rheumatoid synovial fluid are shown in Table I.

Hydrolysate of normal synovial fluid. Anthrone-reacting carbohydrates in the acid hydrolysate of nondialyzable solids of normal fluid were separated into five zones by electrophoresis (Figure 1). Three of these five zones (Zones I, II and IV) had mobilities nearly identical with those of authentic glucose, galactose and mannose. The carbohydrates in the pooled eluates of these zones were studied by chromatography, and glucose,

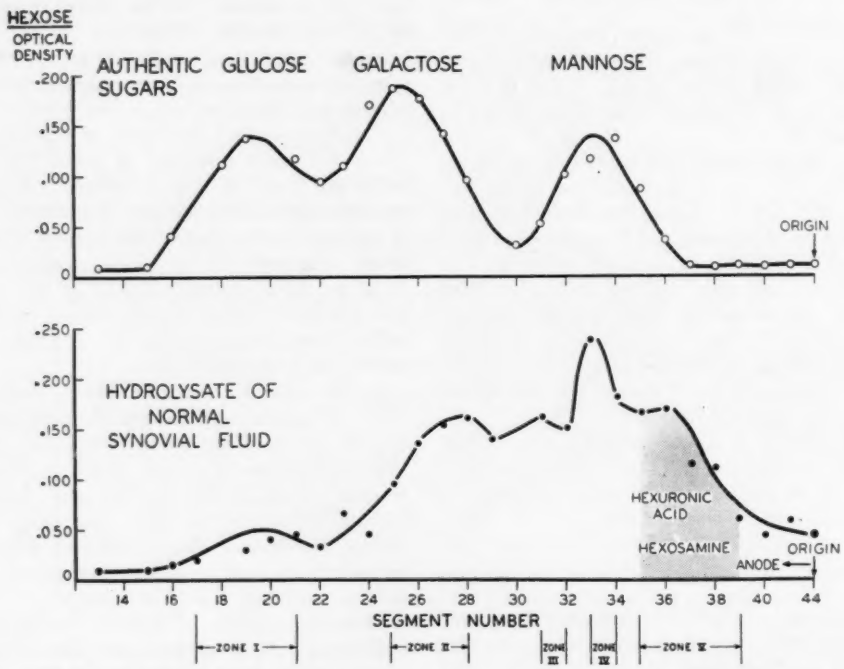

FIG. 1. ZONE ELECTROPHORESIS OF AN ACID HYDROLYSATE OF NONDIALYZABLE SOLIDS OF NORMAL HUMAN SYNOVIAL FLUID, AND OF AUTHENTIC HEXOSES IN BORATE BUFFER, pH 8.5. Eluates were analyzed for carbohydrate with the anthrone reagent and the optical density was plotted for each segment. Eluates within each of five zones were then pooled for chromatographic studies.

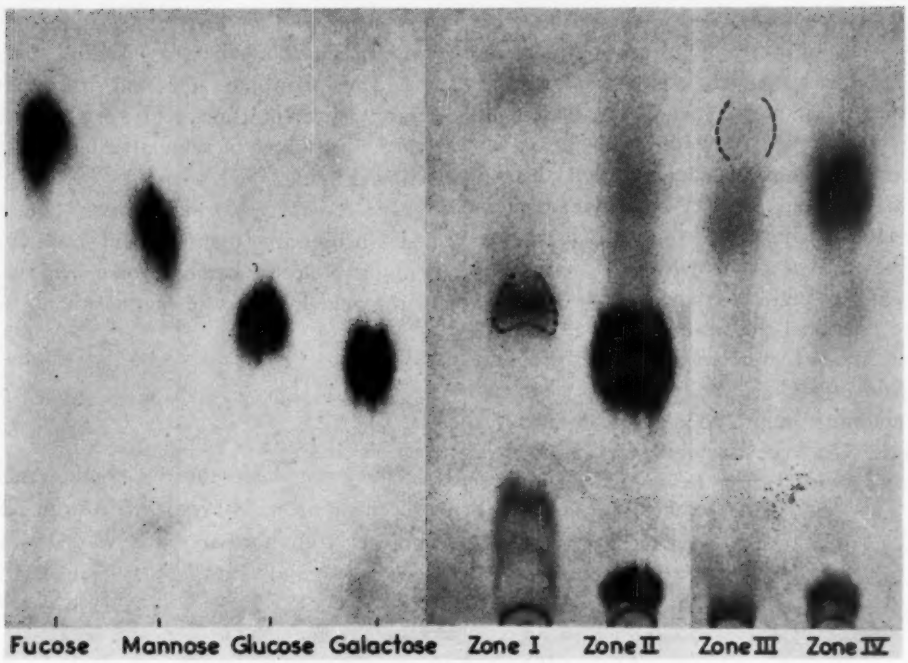

FIG. 2. ASCENDING PAPER CHROMATOGRAM OF HEXOSES IN ZONES I THROUGH IV OBTAINED AFTER ZONE ELECTROPHORESIS, AND OF AUTHENTIC HEXOSES. Isopropyl alcohol-water solvent (8 : 2) ; aniline hydrogen phthalate stain. Zone I, glucose; Zone II, galactose; Zone III, traces of fucose and mannose; Zone IV, mannose.

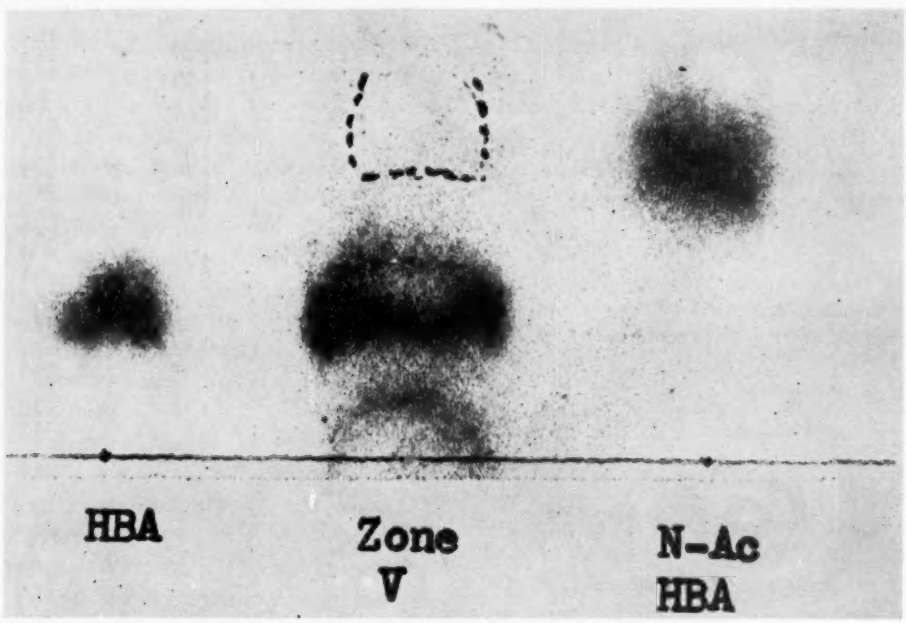

FIG. 3. ASCENDING PAPER CHROMATOGRAM OF HYALURONATE FRAGMENTS IN ZONE V. Butanol-acetic acid-water solvent (6 : 2 : 2) ; *p*-dimethyl aminobenzaldehyde in HCl stain. HBA, hyalobiuronic acid (red) ; N-AcHBA, N-acetyl hyalobiuronic acid (purple). In Zone V the faint purple stain of one component (dotted line) faded; the other component stained red.

galactose and mannose were identified in their respective zones (Figure 2). Fucose was found in Zone III between galactose and mannose.

The fifth zone moved more slowly than mannose, and contained hexosamine and hexuronic acid. After sodium borate was removed, the molar ratio of hexuronic acid to hexosamine in the pooled eluates of this zone was 1. Chromatography revealed two carbohydrates (Figure 3). That present in highest concentration migrated a distance identical to authentic hyalobiuronic acid,[3] and stained red with *p*-dimethylaminobenzaldehyde in HCl. The other sugar, present in trace amounts, migrated a distance identical to authentic N-acetyl hyalobiuronic acid,[3] and stained purple with *p*-dimethylaminobenzaldehyde in HCl.

[3] Kindly supplied by Dr. Karl Meyer, College of Physicians and Surgeons.

Hydrolysates of osteoarthritic and rheumatoid synovial fluids. Glucose, galactose, fucose, mannose, hyalobiuronic acid and trace amounts of N-acetyl hyalobiuronic acid were identified in the acid hydrolysates of nondialyzable solids of these synovial fluids.

Hydrolysate of normal serum. Glucose, galactose, fucose and mannose but no hyalobiuronic acid or N-acetyl hyalobiuronic acid were found in the acid hydrolysate of the ethanol precipitate of serum.

II. Concentration of nondialyzable hexose in synovial fluid

The mean value for the nondialyzable hexose concentration in synovial fluids from 22 deceased subjects with normal joints was 0.54 mg per g (Figure 4 and Table II). Synovial fluid and

TABLE II

Levels of nondialyzable hexose in synovial fluids and sera

		Nondialyzable hexose		Synovial fluid protein
		Synovial fluid	Serum	
		mg/g	*mg/g*	*mg/g*
Normal		0.54 ± 0.11*	1.10 ± 0.09*†	25.3 ± 4.6*
Cerebral vascular accidents	54	0.61	1.90	18.3
	62	0.43	1.40	25.6
	45	0.75	1.56	29.8
	64	0.66	1.67	28.4
	70	0.69	1.68	23.5
Osteoarthritis	Mon	0.35	1.09	23.7
	Gre	0.36	1.20	23.2
	Cuo	0.45	1.15	24.6
	McN	0.53	1.18	26.7
	Tio	0.51	1.04	28.4
	Rag	0.47	1.01	30.4
	Pap	0.56	0.93	26.8
Rheumatoid arthritis	Tim	0.78		
	Cou	0.74		53.7
	Ack	1.06	1.88	62.5
	Mur Lt.	1.06	1.47	50.5
	Rt.	1.25		
	DeG Rt.	0.88	1.47	50.5
	Lt.	0.82		40.7
	Koc	0.91	1.48	
	Cas Rt.	0.78		34.8
	Lt.‡	0.44		
	Mac	0.97		53.5
	Boy Rt.	1.10		63.0
	Lt.‡	0.64		
	Swe	1.07	1.48	46.4
	Nie	1.09	1.75	43.0
	Sco	0.70	1.24	52.5
	Cam Rt.	0.86	1.58	44.0
	Lt.	0.88		44.7

* Standard deviation.
† Analysis of serum from 10 living normal subjects.
‡ Uninvolved knee.

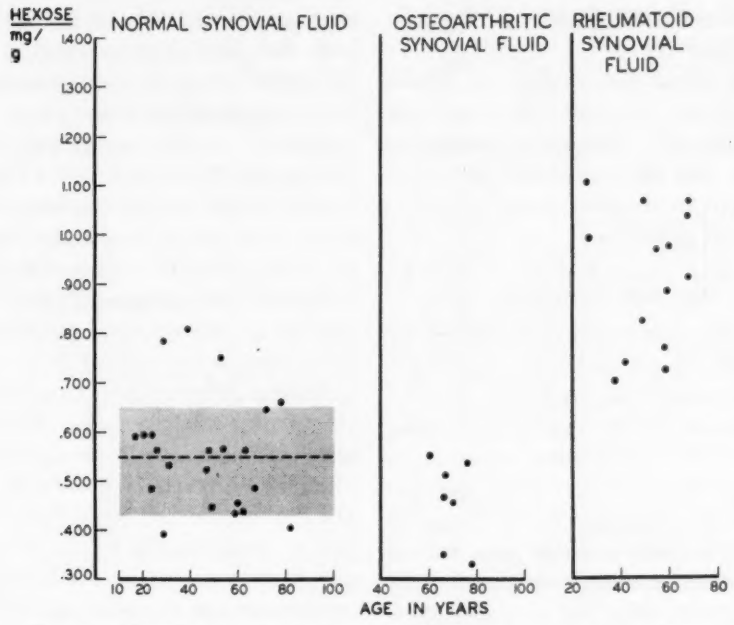

FIG. 4. NONDIALYZABLE HEXOSE CONCENTRATION OF HUMAN SYNOVIAL FLUIDS. The shaded area represents one standard deviation from the mean value (0.54 mg per g) of normal synovial fluid.

serum were also obtained from living, comatose subjects with normal knee joints but with elevated acute phase reactants due to an acute cerebral vascular accident. Although the nondialyzable hexose concentrations in these sera were elevated, hexose concentrations in the dialyzed synovial fluids were normal in four of the five cases.

The nondialyzable hexose concentration was not elevated in seven osteoarthritic fluids (Figure 4 and Table II). The volume of each of these fluids was less than 3 ml except for Tio (50 ml) and Cuo (12 ml). The nondialyzable hexose concentration in the sera of the patients with primary osteoarthritis was normal. The synovial fluid protein concentration was normal in all the osteoarthritic fluids.

The nondialyzable hexose concentration was above 0.70 mg per g in all the rheumatoid fluids from involved knees (Figure 4 and Table II). The nondialyzable hexose concentration in the sera was usually increased. The protein concentration was increased in all the rheumatoid fluids. In two patients with rheumatoid arthritis, synovial fluid was simultaneously obtained from an apparently uninvolved knee [normal physical findings, negative X-ray, volume of synovial fluid less than 2 ml, and normal hyaluronate hexosamine concentration (17)] and from an involved knee. Nondialyzable hexose concentration was normal in the normal knee, and elevated in the involved knee (Table II).

Precipitation of protein-bound hexose by alcohol. Addition of alcohol to synovial fluids or serum precipitated the total protein-bound hexose. Values obtained by this method were similar to

TABLE III

Hexose levels of synovial fluid and serum: comparison of values obtained after dialysis or alcohol precipitation

	Hexose	
	Nondialyzable	Alcohol ppt.
	mg/g	*mg/g*
Synovial fluid:		
Normal	0.79	0.77
	0.43	0.39
	0.60	0.56
	0.46	0.45
Rheumatoid arthritis	1.12	1.19
Serum:		
Normal	1.20	1.19
Rheumatoid arthritis	1.60	1.55
	1.48	1.50

hexose levels obtained after dialysis of synovial fluid or serum (Table III).

Contribution of hyaluronidase-digested hyaluronate to the anthrone reaction. A solution of protein-free hyaluronate fragments containing 400 μg hexuronic acid per ml (1,000 μg hyaluronate per ml) gave an anthrone reaction equivalent to 25 μg per ml of hexose.

DISCUSSION

The carbohydrates in an acid hydrolysate of the nondialyzable solids of synovial fluid were separated by zone electrophoresis on blocks of polyvinyl chloride in borate buffer at pH 8.5. Neutral monosaccharides in the hydrolysates formed negatively charged complexes with borate and migrated in the electric field (18). Good separation of glucose, galactose and mannose was accomplished.[4] Fucose was not completely separated by electrophoresis but was identified by paper chromatography. The use of polyvinyl chloride instead of starch as the supporting medium permitted elution without contamination with carbohydrate.

Galactose, mannose and small amounts of glucose and fucose were identified in the acid hydrolysates of dialyzed, lyophilized synovial fluids and in the ethanol precipitate of serum. The finding of glucose in these hydrolysates is of interest since in a recent discussion glucose was thought not to be one of the hexoses bound to human serum proteins (19). Hyaluronate fragments isolated from the hydrolysates of nondialyzable solids of normal and rheumatoid synovial fluids were identified as hyalobiuronic acid.

The nondialyzable hexose concentration in human synovial fluids was determined with the anthrone reagent using a solution containing equal amounts of galactose and mannose as a standard. The small volumes of viscous fluid taken for analysis were weighed rather than pipetted. The anthrone reaction of isolated protein-free hyaluronate was small (2.5 per cent of the hyaluronate concentration) and, therefore, no correction was made in the hexose levels for the hyaluronate of synovial fluid.

The mean value for the nondialyzable hexose concentration in synovial fluid obtained from subjects with normal joints was 0.54 mg per g. Nondialyzable hexose levels in synovial fluids obtained from osteoarthritic knee joints were normal or decreased. Similar results were found by Decker, McKenzie, McGuckin and Slocumb (2). The nondialyzable hexose concentrations in synovial fluids from joints involved by rheumatoid arthritis were increased. Determination of the nondialyzable hexose concentration in synovial fluid may be a means for differentiating osteoarthritic from rheumatoid synovial fluid.

Studies reported here support the view (20) that the normal synovial membrane selectively adds components of serum to synovial fluid. High serum levels of protein-bound hexose were not accompanied by an increase in the concentration of nondialyzable hexose in synovial fluid obtained from normal joints of patients with acute cerebrovascular accidents and from the uninvolved knees of patients with active rheumatoid arthritis.

There is evidence that nondialyzable hexoses are bound to proteins in synovial fluid (21). Since the protein concentration was elevated in all the rheumatoid fluids, increased hexose levels were expected. However, hexose levels were not always proportional to the increased protein concentration, and it is likely that the very high hexose level in some fluids was due chiefly to an increase in proteins rich in hexose that entered the inflamed joint.

SUMMARY

1. Pooled normal human synovial fluids, and synovial fluids from joints involved by primary osteoarthritis or rheumatoid arthritis were dialyzed and lyophilized. These lyophilized solids were hydrolyzed in acid, and the carbohydrate components were separated by a combination of zone electrophoresis in borate buffer at pH 8.5, and ascending paper chromatography. Glucose, galactose, fucose, mannose, hyalobiuronic acid and traces of N-acetyl hyalobiuronic acid were found in all the hydrolysates.

2. Nondialyzable hexose concentration in normal synovial fluid and fluid from joints involved by primary osteoarthritis or rheumatoid arthritis was determined with the anthrone reagent. A hexose standard containing equal amounts of galactose and mannose was used.

[4] Free hexosamine in the acid hydrolysates was swept off the cathodal end of the block and was not recovered in these studies.

3. The mean value for the nondialyzable hexose concentration in normal synovial fluid was 0.54 mg per g. Osteoarthritic synovial fluids had normal or lower levels, while rheumatoid synovial fluids had values above 0.7 mg per g. Determination of the concentration of nondialyzable hexose may be useful in differentiating osteoarthritic and rheumatoid synovial fluids.

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ABBREVIATION OF BERSON TECHNIC FOR ESTIMATION OF THYROIDAL CLEARANCE OF PLASMA RADIOIODIDE: USE OF BERSON TEST TO RECOGNIZE THYROIDAL PROTEIN-BINDING DEFECTS

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Berson, Yalow, Sorrentino and Roswit (1) designed a simplified technic for estimating the rate at which the thyroid gland clears the plasma of intravenously administered radioiodide (I^{131}). The amount of I^{131} taken up by the thyroid in 30 minutes is correlated with the average concentration of I^{131} in the plasma during the half hour. Plasma I^{131} is not actually measured, but is determined on the assumption that its concentration is a function of the weight of the body. Regardless of this and other assumptions involved, the method is one of the most precise in evaluation of thyroidal function in our experience (2, 3).

Calculation of clearances at periods of time less than 30 minutes has been found necessary on those infrequent occasions when there was an electronic or mechanical failure of the radiation detector, or when the patient was so restless that the uptake tracing became erratic before the 30 minute time was concluded.

In an attempt to determine whether the Berson procedure might be shortened, 1,250 of our records were reviewed. One hundred particularly uniform tracings, with clearance values of not less than 10 to more than 300 ml of plasma cleared of I^{131} per minute, were analyzed at various intervals between 5 and 30 minutes to establish new mathematical constants for practical application of the test.

During the course of our review, 11 tracings were found in which an initial uptake of I^{131} was succeeded, after about 10 or 20 minutes usually, by a reversal of the curve. Not only do these cases present a problem in the calculation of clearance of plasma I^{131}, but their rarity (about 1 per cent) seems to warrant their presentation.

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METHOD

The Berson technic (1) was followed closely, with the only modification consisting of adaptation to our *in vivo* equipment: a 2 inch (5.1 cm) sodium iodide (thallium-activated) scintillation crystal situated at a distance of 20 cm between the surface of the crystal and the skin overlying the thyroid. The radioactivity was registered continuously on a counting rate-meter and in turn on an Esterline-Angus recorder (for example, Figure 1). Pulse-height discrimination was so set that 100 counts per second were recorded per microcurie of I^{131} in the neck.

The equation for calculating the thyroidal clearance (Cl in milliliters cleared per minute), based on the observations of Berson and colleagues, is $Cl = \frac{Th \cdot 200 \cdot W}{\overline{D} \cdot 30}$, where Th is the calculated uptake of I^{131} during the test, W is the body weight in kilograms, $\overline{D}$ is the calculated average fraction of the retained dose of I^{131}, and 30 is the length of test in minutes. Berson's group found a fairly constant ratio between body weight and the fraction of the retained dose of I^{131} in the plasma, a plasma/body weight constant expressed by the number 200 in the above equation.

From the 100 tracings under analysis, the uptake (Th) was calculated at 5, 7.5, 10, 15, 20 and 25 minutes; the time in the denominator was changed accordingly. Assuming Cl from the standard 30 minute test, the only unknown became the plasma/body weight constant; this was then simply calculated.

RESULTS

Calculation of the plasma radioiodide/body weight constant for various intervals of time between 5 and 30 minutes, instead of the conventional 30 minutes, is presented in Table I. The standard deviation is seen to increase as the duration of the test diminishes. However, the error at 20 minutes is sufficiently small that it seems warranted to abbreviate the test routinely to this time from the standpoint of comfort to the patient.

Data on 11 patients with a reversing uptake pat-

tern are included in Table II, and the actual uptake curves are reproduced in Figure 2.

It is clear from inspection of the curves that the standard Berson procedure is inappropriate for the calculation of thyroidal clearance of plasma I^{131} in these cases. This may be shown numerically by calculating the "clearance" at 5 minutes as well as at 30 minutes (Table II). In Patient 5, the one with iodide goiter, "clearances" were found to be 149 ml per minute at 5 minutes, 126 at 10, 108 at 15, 94 at 20, 82 at 25, and only 74 at 30 minutes. Since the diminishing "clearance" values reflect a loss of I^{131} from the thyroid during the test, none of these values truly represents the clearance. The closest approximation was reached by extrapolating the values to time zero, which yielded, in this instance, about 170 ml per minute.

DISCUSSION

The usual pattern of uptake of I^{131} by the thyroid gland is a continuously rising one until secretion of hormonal I^{131} reverses the curve. In normal persons this usually takes place about a day after the dose of I^{131} is given; in an occasional patient with hyperthyroidism, hormonal secretion may begin to reduce thyroidal I^{131} as early as two hours after the dose, although the peak uptake in Graves' disease usually occurs several hours later.

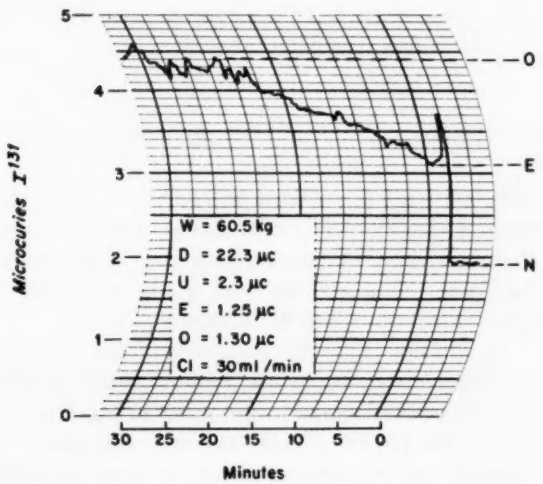

FIG. 1. ERRATIC RECORD OF THYROIDAL UPTAKE OF I^{131}. The patient was restless the last 15 minutes of the test. N, neck background before test started; E, extrathyroidal I^{131} in the neck before significant I^{131} was taken up by thyroid; O, observed uptake of I^{131} during half hour test (actually the difference between value at 30 minutes and value E); W, weight of patient; D, intravenously administered dose of NaI^{131}; U, urinary excretion of I^{131} during test; Cl, thyroidal clearance of plasma I^{131} calculated from these data.

TABLE I

Plasma radioiodide constants (k) in the Berson test of thyroidal clearance of plasma I^{131}, at intervals of 5 to 25 minutes after equilibration in the neck of intravenous doses of I^{131}

Minutes after equilibration	k*	± 1 SD	As % of k	k* Values proposed for routine use
25	191.1	6.9	3.6	190
20	180.0	9.6	5.3	180
15	168.9	13.6	8.1	170
10	157.2	18.4	11.7	160
7.5	147.6	20.3	13.8	150
5	137.3	23.2	16.9	135

* k = Mean value for the constant of plasma I^{131}/body weight. The mean values for k are based on 100 studies of uptake. The value for k at 30 minutes was taken as 200. Berson and associates experimentally determined that in the 30 minute period of their test the average fraction of a dose of I^{131} retained (that is, not in the thyroid or urine) per milliliter of plasma when multiplied by the body weight in kilograms was 5×10^{-3}. In the development of the clearance equation, this value, transposed from the denominator to the numerator, becomes 0.2×10^3 or 200.

In contradistinction to this conventional pattern, 11 patients were observed in whom thyroidal release of I^{131} became evident within the first one-half hour to one hour; it was recognized only because the Berson clearance test was being performed.

Details on the patient with "iodide goiter" have been reported (4). Paris and associates, studying this patient and other patients with "iodide goiter," found that after a rapid initial uptake of I^{131} by the goitrous thyroid, there was a reversal of the uptake curve (4). The rate of disappearance of I^{131} from the thyroid gland exactly matched the rate of disappearance of I^{131} from the blood stream. Since no hormonal I^{131} was recoverable, it would appear that these patients had an adequate or exaggerated iodide-trapping mechanism, but an inability to bind any of the halide to protein. In consequence, an equilibrium between thyroidal iodide and plasma iodide was reached and maintained. Such an equilibrium presumably occurs even when the binding rate of the thyroid is normal (5). Subsequently, the patient was taken off iodide drugs and a fully normal

TABLE II

Eleven patients whose thyroids took up I^{131} initially but tended to release it during period of observation (Figure 2) *

Patient	Age, sex	Diagnosis	Apparent thyroidal clearance of I^{131} at: 5 min	30 min	BMR	Serum PBI	Drugs
			ml/min		%	*μg/100 ml*	
1	24 F	Hashimoto's disease†	47 64	16 13	−23	1.7	Before TSH After TSH
2	33 F	Hashimoto's disease†	33	18	0	4.6	
3	31 F	Hashimoto's disease†	85	49	+5		
4	49 F	Probable Hashimoto's disease	8	3	+6	5.1	
5	33 F	Iodide goiter†	149	74	−11		Iodides‡
6	13 M	Goitrous cretinism†	190	55		2.5	
7	44 F	Mild Graves' disease	93	25	−3	3.9	PTU§
8	58 M	Tension state	17	4	+6	5.0	Tapazole‖
9	39 M	Osteoporosis; hypogonadism	21	4	−14	2.5	PTU¶ TSH
10	66 F	Previous I^{131} therapy for Graves' disease†	13	5			
11	47 F	Smooth, diffuse goiter of uncertain type†	71	22	+18	8.7	

* Abbreviations: BMR = basal metabolic rate; PBI = protein-bound iodine; PTU = propylthiouracil; TSH = thyroid-stimulating hormone.
† Tissue obtained at operation.
‡ For asthma.
§ Received 200 mg 3 times a day for about a year.
‖ Received 10 mg 3 times a day for 3 weeks.
¶ Received 100 mg 3 times a day for 8 days; received also bovine thyrotropin, 5 units daily, for 5 days before test.

function returned to the thyroid gland, including disappearance of the reversing uptake curve.

In the extensive literature on the effect of propylthiouracil, it is agreed that this drug primarily blocks protein binding of thyroidal iodine (6). A reversing uptake curve might, therefore, be expected to occur in patients receiving this drug. Ingbar (7) has proposed an explanation for the rarity of the phenomenon on the basis of the exorbitant doses required to halt the thyroidal binding rate effectively, especially in Graves' disease. He has, however, observed the reversing pattern in patients, as have Taurog, Chaikoff and Feller (8) in animals. Three of our patients were receiving propylthiouracil or methimazole.

Gardner, Hayles, Woolner and Owen (9), in a study of goitrous cretins, found that those patients incapable of converting thyroidal iodine to the protein-bound form were characterized by a reversing uptake curve. The release rate from the thyroid and the renal excretion rate of I^{131} were the same. Our patient with goitrous cretinism would seem to fit into this category.

By analogy with the various types of patients described above (goitrous, cretins, iodide goiter, propylthiouracil goiter), the same defect characterized the patient (Table II) who had had I^{131} therapy for hyperthyroidism, the four patients with Hashimoto's disease and the one with an undiagnosed goiter.

From Ingbar's data it is difficult to explain the findings in Hashimoto's disease; he estimated that the thyroidal binding rate had to be inhibited 90 per cent to obtain a "negative slope accumulation curve" (7). The level of hormonal iodine, i.e., butanol-extractable iodine and butanol-insoluble

iodine (10) in the plasma of these patients often totals a normal value for protein-bound iodine (for example, Patients 2 and 4), suggesting a binding rate considerably greater than 10 per cent of normal. Although our experience with the Berson test in Hashimoto's disease is limited to these four patients, the unanimity of the finding of a reversing uptake curve suggests a new diagnostic clue.

The eleventh patient is difficult to classify, because we are not certain what type of goiter she had. She was 47 years old, with mitral stenosis and insufficiency, and was found to have a small, diffuse, non-nodular goiter. She was euthyroid clinically. The basal metabolic rate was plus 18 per cent. The protein-bound iodine was increased (8.7 μg per 100 ml) but the uptake of I^{131} was not commensurate (13 per cent in one-half hour and 6 per cent in six hours, with only about 1 per cent remaining after 24 hours). No explanation for this discrepancy between protein-bound iodine and I^{131} uptake was found. No history of ingestion of interfering medication could be obtained. Needle biopsy of the thyroid showed microfollicles and macrofollicles, but no thyroiditis or parenchymatous hypertrophy.

SUMMARY AND CONCLUSIONS

One hundred tracings of thyroid uptake were analyzed at intervals of 5, 7.5, 10, 15, 20 and 25 minutes after extrathyroidal equilibration of intravenously administered I^{131}, to establish constants for calculations of thyroidal clearance of plasma I^{131} by the method of Berson and colleagues.

In the Berson calculation, a constant of 200 for the plasma I^{131}/body weight relationship was proposed for the usual 30 minute interval. Our calculated constants for shorter intervals were: 137.3 ± 23.2 (1 SD) at 5 minutes; 147.6 ± 20.3 at 7.5 minutes; 157.2 ± 18.4 at 10 minutes; 168.9 ± 13.6 at 15 minutes; 180.0 ± 9.6 at 20 minutes; and 191.1 ± 6.9 at 25 minutes. For clinical purposes the accuracy is sufficient at 20 minutes, so that we recommend shortening the test on a routine basis to 20 minutes, since the test is most useful in those patients least able to lie quietly, that is, those with Graves' disease.

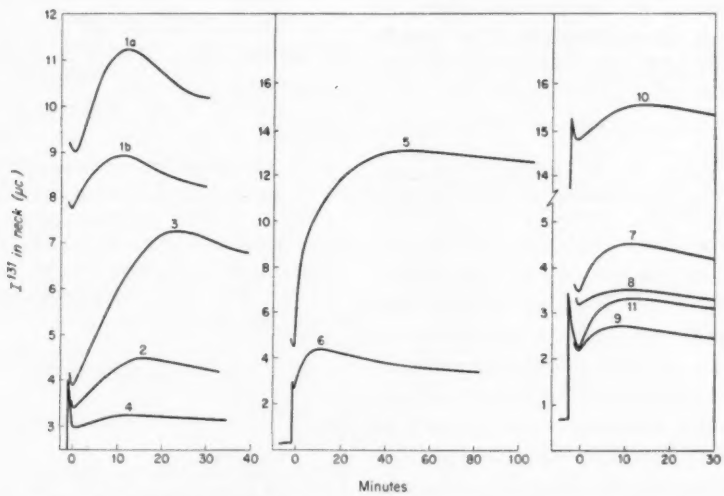

FIG. 2. THYROIDAL UPTAKE OF I^{131} IN 11 PATIENTS WHO CONCENTRATED I^{131} IN THEIR THYROIDS INITIALLY BUT SUBSEQUENTLY RELEASED PART OF IT (TABLE II). Uptake is recorded in microcuries of I^{131}. The abscissa is time in minutes after equilibration of the I^{131} in the neck, that is, before actual thyroidal uptake has begun. The test was performed twice on Patient 1: 1a, before giving thyroid-stimulating hormone, and 1b, 24 hours after intramuscular injection of 10 units of bovine thyroid-stimulating hormone. Doses of I^{131} given Patient 1a and 1b were 50.0 and 25.0 μc, respectively. Patients 2 through 11 received 23.8, 23.5, 50.5, 52.0, 14.7, 23.8, 22.3, 50.4, 51.5, and 22.4 μc., respectively.

With short-time constants available, the Berson clearance test may be abbreviated as needed for the convenience of the patient, or as required if the equipment should fail before the standard test is concluded, or as needed for evaluation of the rare patient who traps I^{131} but releases it promptly, presumably still in its inorganic form.

Eleven patients, from among 1,250 studied, exhibited the phenomenon of normal or accelerated thyroidal uptake of I^{131} followed, within the half hour of the Berson test, by reversal of the uptake curve. One patient was a goitrous cretin, one had an iodide goiter, three were receiving propylthiouracil or methimazole (Tapazole), and one had been treated with a therapeutic dose of I^{131}. In these six patients, apparently complete block in thyroidal protein-binding of iodine existed; the loss of thyroidal radioiodide reflected the equilibrium between thyroidal and plasma iodide, an equilibrium dominated by the renal excretion of iodide.

The remaining five patients, four with Hashimoto's disease and one with a goiter of undiagnosed type, also exhibited a reversal of I^{131} uptake during the Berson test. Here the explanation is less clear; serum values for protein-bound iodine do not warrant the assumption of a prominent defect of protein-binding of iodine in the thyroid.

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NON-UNIFORM PULMONARY DIFFUSION AS DEMONSTRATED BY THE CARBON MONOXIDE EQUILIBRATION TECHNIQUE: MATHEMATICAL CONSIDERATIONS*

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It is now well established that both ventilation and perfusion are distributed unevenly throughout the lung. Inert gas washout curves have consistently revealed non-uniformity of ventilation/lung volume ($\dot{V}_A/V_A$) ratios (1–3); study of the respiratory exchange ratios in different parts of the lung has provided evidence of uneven ventilation/perfusion ($\dot{V}_A/\dot{Q}_C$) ratios (4, 5), and both bronchospirometric studies (6) and measurements of physiological dead space (7) have supported these findings.[1] Analysis of single expirates has indicated, in addition to $\dot{V}_A/\dot{Q}_C$ inequality, non-uniformity of perfusion/lung volume ratios ($\dot{Q}_C/V_A$) throughout the lung (8, 9). Results with differential bronchiospirometry indicate a definite $\dot{Q}_C/V_A$ difference between the two lungs when the subject lies on his side (10). Available evidence suggests that perfusion is reduced in the superior portions of lung due to hydrostatic effects (11), accounting for an increased unevenness of $\dot{V}_A/\dot{Q}_C$ in the erect posture as compared with the supine (7) and explaining the low oxygen uptake and low $\dot{Q}_C/V_A$ ratio of the uppermost portions of the lung (6, 10, 12). One would expect diffusion across the alveolar-capillary membrane in a given volume of lung to be related to the effective capillary bed in that region, and variation in $\dot{Q}_C/V_A$ to be reflected in variations of the ratio of diffusing capacity to lung volume (D_L/V_A).

Direct evidence of D_L/V_A non-uniformity was first reported by Forster, Fowler, Bates and Van Lingen (13). These investigators noted that the disappearance of carbon monoxide from alveolar gas during breath holding did not follow a simple exponential curve as theoretically expected; the effect was explained by non-uniform D_L/V_A ratios in the lung, those areas with low diffusion causing an apparent slowing of carbon monoxide disappearance when long breath holding times were utilized. The findings of Forster and co-workers have been confirmed by others (14, 15) although at least one observer has failed to note the phenomenon (16). Unfortunately, the breath holding method is not suitable for quantification of non-uniformity of D_L/V_A; with this method, overall D_L is esti-

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[1] Definitions of symbols:

D_L = pulmonary diffusing capacity
V_A = alveolar volume
$\dot{V}_A$ = alveolar ventilation per unit time
$\dot{Q}_C$ = pulmonary capillary blood flow
V_{TA} = alveolar tidal volume
V_{FRC} = end expiratory alveolar volume
V_C = blood volume of the pulmonary capillary bed
D_M = true diffusing capacity of the pulmonary membrane
t = any time interval
tb = time for a single breath cycle
f = respiratory frequency (f = 1/tb)
P_B = barometric pressure corrected for water vapor
Y and Z = symbols for compartments of the lung, applied as subscripts (e.g., V_{AZ} and so forth)
F_{AX} = fractional concentration of any gas X in alveolar gas
F_{IX} = fractional concentration of any gas X in inspired gas
F_{ETX} = fractional concentration of any gas X in end tidal gas
F_{EIX} = fractional concentration of any gas X in alveolar gas at the end of inspiration
$F\dot{V}_Y$ = fraction of $\dot{V}_A$ to compartment Y
$F\dot{V}_Z$ = fraction of $\dot{V}_A$ to compartment Z
K_{N_2} = a constant indicative of the rate of inert gas washout
K_{CO} = a constant indicative of the total rate of carbon monoxide disappearance from alveolar gas due to diffusion plus ventilation
K_D = a constant indicative of the rate of carbon monoxide disappearance from alveolar gas due to diffusion alone ($K_D = P_B D_L/V_A$)
W = the dilution factor for a uniform lung during washout, such as $W = V_{FRC}/(V_{FRC} + V_{TA})$
Subscript t = at time t
Subscript 0 = at onset of time interval t
Subscript ∞ = after complete equilibration with an inhaled gas.

mated by using an arbitrary period of breath holding and assuming uniform diffusion. Thus, calculated D_L varies with the period of breath holding utilized. Other methods for determining the pulmonary diffusing capacity [e.g., the steady state carbon monoxide method of Filley, MacIntosh and Wright (17) and the oxygen method of Riley and Cournand (18)] do not directly reflect variability in D_L/V_A but may yield erroneous values for total D_L in the presence of $D_L/\dot{V}_A$ or $\dot{V}_A/\dot{Q}_C$ non-uniformity. This problem has been reviewed in some detail by Forster (19).

In 1958, a method was described in this laboratory for determining D_L from carbon monoxide equilibration curves (20), a method which was similar in approach to that of Gilson and Hugh-Jones (21). With the technique originally employed, no evidence of D_L/V_A variability was demonstrated in several normal individuals. However, with improved methods (including more careful control of tidal volume and breathing pattern as well as incorporation of helium as an indicator of inert gas equilibration), it is apparent that D_L/V_A ratios are non-uniform in the normal subject. In addition, it has been noted that unevenness of diffusion may be increased by full inflation of the lung (15), and (as will be shown in the following paper of this series) that D_L/V_A variations may be marked in pathological lungs and in certain experimental preparations. The equilibration technique provides sufficient data to calculate D_L/V_A variations if certain assumptions are allowed. It is the purpose of this report to describe the theoretical basis for such calculations and to outline the methods involved. In addition to assessing the degree of non-uniformity of diffusion, such methods could provide an estimate of overall D_L which is free from the errors induced by non-uniformity of $\dot{V}_A/V_A$, $\dot{Q}_C/\dot{V}_A$, or D_L/V_A. In the second paper of this series, these methods of calculation are applied to obtain experimental data in man.

CALCULATIONS

Mathematical relationships in a uniform lung. It is possible to predict the changes in end tidal gas which will result in an ideal uniform lung when inhaled gas is changed from room air to a helium-carbon monoxide mixture and when this mixture is replaced by room air. The mathematical relationships during carbon monoxide equilibration and washout were considered in some detail in a previous report (20). In the ideal lung there will be an exponential increase in $F_{ET_{He}}$ following exposure to helium, and at equilibrium $F_{ET_{He_\infty}} = F_{I_{He}}$. On inspiring the carbon monoxide-helium mixture, there will be an exponential increase in end tidal carbon monoxide, but in the presence of diffusion, $F_{ET_{CO_\infty}} < F_{I_{CO}}$. When room air is again inhaled, $F_{ET_{CO}}$ and $F_{ET_{He}}$ will decrease exponentially to baseline values.

The rate of decline of carbon monoxide must exceed that of helium since carbon monoxide disappears from the alveoli due to a combination of diffusion and simple washout, while helium disappears solely from ventilatory effects. Since both helium and carbon monoxide disappearances will follow a simple exponential function during washout, successive end tidal values of each will describe a straight line when plotted semilogarithmically against time. In this circumstance, D_L may be calculated simply from obtained data if V_A is known:

$$F_{ET_{He_t}} = F_{ET_{He_0}} e^{-K_{N_2} t}, \quad (1)$$

$$F_{ET_{CO_t}} = F_{ET_{CO_0}} e^{-K_{CO} t}, \quad (2)$$

and

$$D_L = V_A(K_{CO} - K_{N_2})/P_B, \quad (3)$$

Equations 1 and 2 defining the constants K_{N_2} and K_{CO}.

In addition, it was shown (20) that

$$F_{A_{CO_\infty}} = F_{I_{CO}} K_{N_2}/K_{CO}. \quad (4)$$

If one considers the changes in $F_{A_{CO}}$ during a single breath cycle after equilibration with an inspired CO mixture (Figure 1), it will be noted that $F_{A_{CO_\infty}}$ calculated from Equation 4 is an "average" value and that $F_{A_{CO_\infty}} \neq F_{ET_{CO_\infty}}$ unless $f = \infty$. In order to utilize the observable $F_{ET_{CO_\infty}}$ in place of $F_{A_{CO_\infty}}$ in Equation 4, a relationship must be derived between $F_{A_{CO_\infty}}$ and $F_{ET_{CO_\infty}}$. With the ventilatory pattern described in Figure 1, and when $F_{A_{CO_\infty}} = 0.5F_{I_{CO}}$,

$$F_{A_{CO_\infty}} = (F_{EI_{CO_\infty}} + F_{ET_{CO_\infty}})/2. \quad (5)$$

Equation 5 is strictly true only when $F_{A_{CO_\infty}} = 0.5F_{I_{CO}}$, because only then will the $F_{A_{CO}}$ curves during inspiration and expiration be mirror images of one another. Since there is no change in $F_{A_{CO}}$ due to ventilation in the ex-

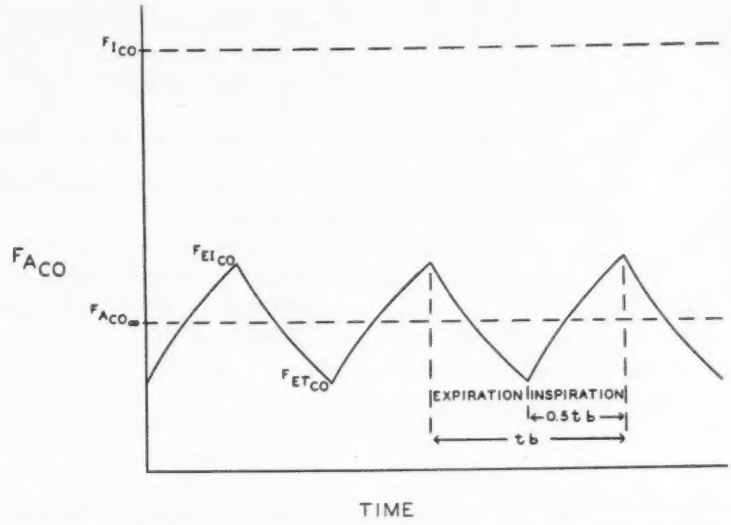

FIG. 1. CHANGES IN THE CONCENTRATION OF CARBON MONOXIDE IN ALVEOLAR GAS (F_{ACO}) AFTER EQUILIBRATION WITH AN INSPIRED CO MIXTURE DURING THE BREATHING CYCLE. It is assumed that inspiration proceeds at such a rate that there is an exponential increase in F_{ACO} during the inspiratory period (i.e., during inspiration, $dF_{ACOt} = dtK(F_{ICO} - F_{ACOt})$. The peak value of F_{ACO} will be reached at the end of inspiration (F_{EICO_∞}). During expiration, loss of carbon monoxide due to diffusion results in a decreasing F_{ACO}, reaching its lowest value at end expiration (F_{ETCO_∞}). Inspiration and expiration are assumed to be equal in duration.

piratory phase, by Krogh's formula (22),

$$F_{EICO_\infty}/F_{ETCO_\infty} = e^{P_B D_L 0.5tb/V_A}.$$

Since

$$K_D = P_B D_L/V_A,$$

$$F_{EICO_\infty}/F_{ETCO_\infty} = e^{K_D 0.5tb}.$$

Combining this last equation with Equation 5,

$$F_{ETCO_\infty} = 2F_{ACO_\infty}e^{-0.5tbK_D}/(1 + e^{-0.5tbK_D}). \quad (6)$$

When $F_{ACO_\infty} \neq 0.5F_{ICO}$, the estimate of F_{ACO_∞} in Equation 5 is inexact, and this error is reflected in Equation 6. However, the final error is small unless F_{ACO_∞} approaches either zero or F_{ICO}; this is unlikely to occur with physiological parameters for K_{N_2} and K_{CO}.

Since it has been shown (20) that $K_D = K_{CO} - K_{N_2}$, it is now possible to approximate F_{ETCO_∞} from Equations 4 and 6 if these K values are known. These relationships will be necessary in the calculation of a two-compartment system to be discussed.

Mathematical relationships in a two-compartment lung. When successive end tidal values for an inert insoluble gas during washout fail to define a straight line when plotted similogarithmically against time, variability of $\dot{V}_A/V_A$ is indicated. If such data are suitable for analysis in terms of a two-compartment system,

$$F_{ET_{He_t}} = F\dot{V}_Y F_{ET_{He_{oY}}}e^{-K_{N_{2Y}}t} + F\dot{V}_Z F_{ET_{He_{oZ}}}e^{-K_{N_{2Z}}t}. \quad (7)$$

Since

$$F_{ET_{He_{oY}}} = F_{ET_{He_{oZ}}} = F_{ET_{He_o}},$$

obtainable data define all values in Equation 7, and, by graphic analysis, values for $F\dot{V}_Y$, $K_{N_{2Y}}$, $F\dot{V}_Z$, and $K_{N_{2Z}}$ may be determined. Also, the slope of the washout points determines W_Y and W_Z (see analysis of experimental data). Since total V_{FRC} may be determined independently, it is now possible to calculate V_{FRC_Y}, V_{FRC_Z}, V_{TA_Y}, and V_{TA_Z} by modification of the methods of Fowler, Cornish and Kety (2):

$$V_{FRC_Y}/V_{FRC_Z} = F\dot{V}_Y W_Y(1 - W_Z)/F\dot{V}_Z W_Z(1 - W_Y), \quad (8)$$

$$V_{FRC_Z} = \frac{V_{FRC}}{(V_{FRC_Y}/V_{FRC_Z}) + 1}, \quad (9)$$

$$V_{FRC_Y} = V_{FRC} - V_{FRC_Z}, \quad (10)$$

$$V_{TA_Y} = V_{FRC_Y}(1 - W_Y)/W_Y, \quad (11)$$

$$V_{TA_Z} = V_{FRC_Z}(1 - W_Z)/W_Z. \quad (12)$$

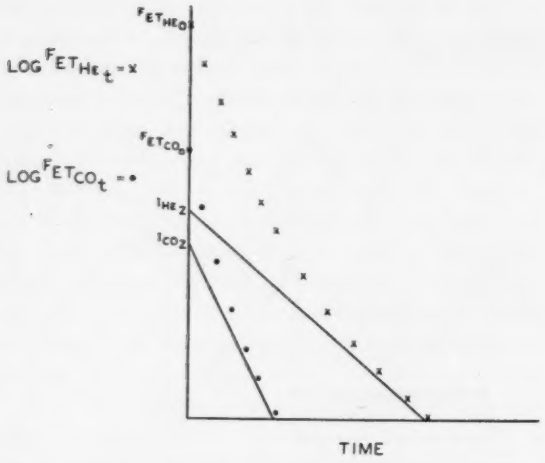

FIG. 2. HYPOTHETICAL END TIDAL CARBON MONOXIDE AND HELIUM CONCENTRATIONS AS MIGHT BE OBTAINED IN THE WASHOUT PERIOD OF AN EQUILIBRATION TEST, PLOTTED SEMILOGARITHMICALLY AGAINST TIME. The oblique solid lines represent tangents to the terminal portions of the curves determined by these successive helium and carbon monoxide values. See text for further explanation.

As discussed in a previous report (20), V_A may be approximated as $V_{FRC} + V_{TA}/2$, allowing calculation of V_{A_Y} and V_{A_Z} from the above parameters:

$$V_{A_Y} = V_{FRC_Y} + V_{TA_Y}/2, \quad (13)$$

$$V_{A_Z} = V_{FRC_Z} + V_{TA_Z}/2. \quad (14)$$

Successive end tidal carbon monoxide values will fail to define a straight line on semilogarithmic plot if K_{CO}'s are non-uniform throughout the lung. Since $K_{CO} = K_{N_2} + K_D$, this could be due to non-uniformity of K_{N_2}, K_D, or both. Since $K_D = P_B D_L/V_A$, non-uniformity of K_D implies unevenness of D_L/V_A ratios. If data may be expressed in terms of two compartments, Y and Z, $F_{ET_{CO_t}}$ will follow a modification of Equation 2:

$$F_{ET_{CO_t}} = \dot{F}V_Y F_{ET_{CO_{0_Y}}} e^{-K_{CO_Y} t} + \dot{F}V_Z F_{ET_{CO_{0_Z}}} e^{-K_{CO_Z} t}. \quad (15)$$

Obtained carbon monoxide washout curves will not provide a unique solution to this above equation since $F_{ET_{CO_{0_Y}}} \neq F_{ET_{CO_{0_Z}}} \neq F_{ET_{CO_0}}$. In this important respect, there is considerable difference in the complexity of Equations 7 and 15. Since K_{CO_Y} and K_{CO_Z} cannot be determined directly from Equation 15, it will be necessary to utilize the additional relationships in each compartment at time ∞. To this end, Equations 4 and 6 may be rewritten to apply to the individual compartments Y and Z. Also,

$$F_{ET_{CO_\infty}} = \dot{F}V_Y F_{ET_{CO_{\infty_Y}}} + \dot{F}V_Z F_{ET_{CO_{\infty_Z}}}. \quad (16)$$

When applied simultaneously to obtained data, the relationships in Equations 4, 6, 15 and 16 allow a solution of the diffusing characteristics of Y and Z if certain assumptions are utilized.

Analysis of experimental data in terms of a two-compartment model. It will be assumed that the lung may be defined in terms of a simple model with a specific number of ventilatory and diffusing compartments and that these compartments may be related to each other in some predetermined manner. In order to minimize calculated variations in D_L/V_A, those alveoli with the slowest ventilation (lowest K_{N_2}) are assumed also to have the slowest ventilation plus diffusion (lowest K_{CO}). This implies that the slowest portions of both helium and carbon monoxide washout curves arise from the same alveoli.

Consider an equilibration test yielding the data in Figure 2. Successive end tidal helium values fail to define a straight line when plotted logarithmically against time, indicating variable $\dot{V}_A/V_A$ ratios. In a two-compartment lung, the lower $\dot{V}_A/V_A$ ratio (Compartment Z) will be reflected in the slope of the terminal portion of the helium curve. A tangent to this portion of the curve has a slope equal to log W_Z. $K_{N_{2_Z}}$ may be determined from two points on the tangent as follows:

$$K_{N_{2_Z}} = \ln (F_{ET_{He_{0_Z}}}/F_{ET_{He_{t_Z}}})/t.$$

The intercept of the inscribed tangent at zero time (I_{He_Z}) will determine $\dot{F}V_Z$:

$$\dot{F}V_Z = I_{He_Z}/F_{ET_{He_0}}. \quad (17)$$

Subtracting the inscribed tangent from successive end tidal helium values allows one to draw a line for Compartment Y; its slope represents log W_Y. From this slope, $K_{N_{2_Y}}$ may be determined:

$$K_{N_{2_Y}} = \ln (F_{ET_{He_{0_Y}}}/F_{ET_{He_{t_Y}}})/t.$$

$\dot{F}V_Y$ equals $1 - \dot{F}V_Z$ in a two-compartment lung. If V_{FRC} is known, the values for $\dot{F}V_Y$, $\dot{F}V_Z$, W_Y, and W_Z may be utilized in Equations 8 through 12 to determine V_{FRC_Y}, V_{FRC_Z}, V_{TA_Y}, and V_{TA_Z}.

Then, V_{A_Y} and V_{A_Z} are calculated from Equations 13 and 14.

Now consider the carbon monoxide curve in Figure 2. Using the lung model defined, the terminal slope of this curve must represent the washout plus diffusion in Compartment Z. If the lung model described is applicable and contains only two compartments, it must be possible to inscribe a tangent to the terminal slope of the carbon monoxide washout curve which will yield a value for $F\dot{V}_Z$ identical with that determined from helium data in Equation 17. The slope of such a tangent would determine K_{CO_Z}:

$$K_{CO_Z} = \ln (F_{ET_{CO_{0_Z}}}/F_{ET_{CO_{t_Z}}})/t,$$

and

$$K_{D_Z} = K_{CO_Z} - K_{N_{2_Z}}.$$

Since the carbon monoxide in Compartment Y approximates zero by the time the final end tidal CO values are obtained, the intercept of the above described tangent at zero time (I_{CO_Z}) would determine $F\dot{V}_Z$, if $F_{ET_{CO_{0_Z}}}$ were known. $F_{ET_{CO_{0_Z}}}$ does not equal observed $F_{ET_{CO_0}}$, but may be calculated by applying values for $K_{N_{2_Z}}$, K_{D_Z}, and K_{CO_Z} to Equations 4 and 6. $F_{ET_{CO_\infty}}$ from these equations is equal to $F_{ET_{CO_{0_Z}}}$ for the carbon monoxide washout period. Having determined $F_{ET_{CO_{0_Z}}}$,

$$F\dot{V}_Z = I_{CO_Z}/F_{ET_{CO_{0_Z}}}. \qquad (18)$$

If the values for $F\dot{V}_Z$ from Equations 17 and 18 do not agree, it may be possible to adjust the tangent to the terminal carbon monoxide points until agreement is reached. The ability to inscribe a satisfactory tangent is a test of the suitability of a two-compartment lung model. When agreement in $F\dot{V}_Z$ has been achieved, the value of $F_{ET_{CO_{0_Z}}}$ may be utilized in determining the parameters of Compartment Y as follows:

$$F_{ET_{CO_{0_Y}}} = (F_{ET_{CO_0}} - F\dot{V}_Z F_{ET_{CO_{0_Z}}})/F\dot{V}_Y.$$

Substituting $F_{ET_{CO_{0_Y}}}$ and $K_{N_{2_Y}}$ in Equations 4 and 6 allows calculation of K_{CO_Y} and K_{D_Y}.

Now,

$$D_{L_Y} = K_{D_Y} V_{A_Y}/P_B,$$

$$D_{L_Z} = K_{D_Z} V_{A_Z}/P_B,$$

and

$$D_L = D_{L_Y} + D_{L_Z}.$$

Since values for Compartment Y were determined entirely from relationships at time ∞, it is still necessary to demonstrate that these parameters satisfactorily explain the end tidal carbon monoxide values during washout. To this end, obtained values for both compartments are applied to Equation 15, and the theoretical carbon monoxide disappearance curves for Compartments Y + Z are compared with obtained end tidal points. Agreement confirms the suitability of the model utilized and serves as a check on calculations.

If a satisfactory solution cannot be obtained in terms of two compartments, a new model must be utilized in which three or more compartments are calculated. The methods are identical to those described above, the contributions of the additional compartments being subtracted serially from observed data.

DISCUSSION

In the method outlined, an attempt is made to analyze data obtained during equilibration and disequilibration with a carbon monoxide mixture in terms of a compartmented lung. It has been found necessary to utilize an arbitrary lung model, the suitability of which must be proven by its applicability to data obtained. Such a model does not pretend to reflect actual anatomic divisions of the lung. However, the description of non-uniform events in terms of simple compartments has been widely utilized, and the compartment concept greatly simplifies calculations.

It has been necessary to assume a relationship between ventilatory and diffusing compartments in order to keep the number of compartments at a minimum. There is experimental evidence that D_L/V_A ratios are similar in portions of the lung which vary in $\dot{V}_A/V_A$, at least as $\dot{V}_A/V_A$ variations are reflected in various portions of expired gas (23). Despite this report, it is difficult to believe that D_L/V_A ratios bear no relation whatever to ventilatory characteristics, at least in pathological lungs (e.g., lung cysts). In any event, applying non-uniform D_L/V_A ratios to each ventilatory compartment would create a minimum of four compartments. In addition, such a model would tend to exaggerate D_L/V_A unevenness in certain situations. By assuming that slowly ventilated areas have a

slow sum of ventilation plus diffusion, one minimizes calculated variations in D_L/V_A and allows the determination of uniform diffusion in the face of $\dot{V}_A/V_A$ variability. It would be impossible to assume identity of a fast ventilating region (Y) with one having slow ventilation plus diffusion (Z) since, in some sets of data, $K_{N_{2Y}}$ exceeds K_{CO_Z}. Support for the approach used may be found in the observations of West, Fowler, Hugh-Jones and O'Donnell (9) who noted that poorly ventilated alveoli appear to be poorly perfused.

An assumption has been utilized in derivation of Equation 6; this formula is based on a breathing pattern in which inspiration and expiration are equal in time and in which inspiration proceeds at such a rate that there is an exponential increase in F_{ACO} during the inspiratory period. Any other breathing pattern could have been utilized as a basis for calculation, but the one employed is simple and closely resembles physiological conditions. The error involved in minor deviations from this pattern is small.

In the method described, there is the tacit assumption that end tidal samples are representative of the alveolar pool at that point in time. When there is uniformity of the expired gas throughout the expiratory period, this assumption is valid, and this situation is approached for an inert gas in a normal subject. With an abnormal lung, one will tend to overestimate the size of the slowly ventilated areas by end tidal samples, since such areas tend to empty relatively late in expiration. Ogilvie, Forster, Blakemore and Morton have been unable to relate diffusion measurements to the portion of expirate examined (23); if this is the case, diffusion measurements in end tidal samples should be valid for the lung as a whole. However, the sizes of the various compartments might still be in error in the abnormal lung since these dimensions are dependent upon inert gas measurements. Besides, it is difficult to believe that Forster's observations of equal diffusion in all portions of the expirate would apply universally. The errors involved in end tidal sampling could be circumvented by analysis of mixed single expirates; as yet, this is not technically feasible.

It should also be emphasized that the analytical methods described herein are crude and time consuming, consisting of a trial and error graphic fit. It seems likely that refinements in technique of calculation are possible, especially by incorporation of electronic computation. This might allow the utilization of a more complex lung model which would more closely resemble physiological parameters.

The method described is subject to all the assumptions and errors of carbon monoxide techniques in general. During the early phases of carbon monoxide disappearance, the problems are similar to those of the breath holding method; these have been discussed in detail by Forster and co-workers (13). As one approaches the terminal portion of the carbon monoxide disappearance curve, the problem of back pressure becomes more important. Ideally, one would account for the value of the back pressure of carbon monoxide in formulation of diffusion effects, but this would add considerably to the complexity of calculations. As the procedure is currently employed, the final $F_{ET_{CO}}$ achieved after several minutes of air breathing is utilized as a baseline for $F_{ET_{CO_t}}$ during washout and, as such, represents an estimate of CO back pressure. This value for $F_{ET_{CO}}$ is a result of two opposing phenomena—the back diffusion of carbon monoxide from blood to alveolus and the ventilation of carbon monoxide from alveolus to atmosphere. The final value of $F_{ET_{CO}}$ will lie somewhere between the carbon monoxide in room air and the tension of carbon monoxide in the pulmonary capillary blood. Due to this inaccuracy in estimation of true back pressure, some error in calculated K_{CO} must result. With normal parameters, and with low concentration of carbon monoxide in inhaled gas, this error is relatively small.

Despite the various limitations outlined, the ability to estimate total pulmonary diffusing capacity in the face of variable D_L/V_A ratios would seem of sufficient importance to warrant a trial of the technique, even if quantitative technics are imperfect. In a following paper these methods are applied to data obtained in human subjects. Animal experiments are also reported which indicate the validity and applicability of the procedure.

SUMMARY

1. A method has been described for calculating compartments with varying diffusing character-

istics (i.e., non-uniform diffusing capacity to lung volume ratios) from carbon monoxide equilibration curves. By adding the diffusing capacities calculated for each compartment, a total diffusing capacity may be determined which is theoretically free of the errors induced in conventional diffusion techniques by non-uniformity of pulmonary diffusion.

2. The methods described require the utilization of an arbitrary lung model. The applicability of this model must be demonstrated by its ability to explain experimental results.

3. In the following paper, these methods will be applied to experimental data, and further implications of diffusing capacity per lung volume non-uniformity will be discussed.

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THE DIALYSANCE OF ETHANOL AND METHANOL: A PROPOSED METHOD FOR THE TREATMENT OF MASSIVE INTOXICATION BY ETHYL OR METHYL ALCOHOL *

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(Submitted for publication November 5, 1959; accepted January 29, 1960)

Previous studies from this laboratory (1) have cited a time-dose-cytotoxic relationship and a high dialysance [1] as important theoretical criteria for the selection of dialyzable poisons. Small molecular size, high solubility, clinical correlation with blood level and a primary distribution in the fluid phase of the body would suggest ethanol and methanol as ideal dialyzable toxins. Because of the time lag between ingestion of methanol and production of its lethal metabolites such as formic acid, early dialysis would seem critical in cases of methanol poisoning. Since each metabolite of methanol is more toxic, removal of the parent compound offers a unique leverage and constitutes preventive medicine as well as specific therapy.

METHOD

The dialysance of both alcohols was studied *in vitro* on the twin-coil artificial kidney [2] using an 18 L reservoir which contained ethanol or methanol dissolved in plasma or normal saline. Since no inhibition of dialysance could be detected by the addition of protein, most experiments were conducted in saline. The solutions were circulated against a 100 L bath of fluid containing an equiosmolar concentration of solutes excluding the alcohol, at various flow rates measured at the venous end of the system by volume determinations of a timed collection. Once the coil was reasonably distended, outflow pressure (measured from the bubble catcher) was maintained constantly at zero. An interval of one circulation time was allowed between arterial and venous samplings, while bath samplings took place half-way between the two measurements.

In vivo experiments were carried out on 11 female mongrel dogs ranging in weight from 13.1 to 27.2 kg. Absolute spirits in doses of 3.2 to 6 ml per kg were infused intravenously as a 20 per cent solution in saline over a period of 30 to 60 minutes. Each set of experiments included 3 animals given equal amounts of alcohol; 2 of them were observed as controls against a third animal submitted to hemodialysis. One experiment was conducted with a single control.

Extracorporeal circulation was established from the left femoral artery through a single coil of the conventional twin-coil dialyzer and returned to the animal via the adjacent femoral vein. A solution of 6 per cent dextran and 10 per cent invert sugar in saline was used to prime the machine. The right femoral artery was also cannulated and connected to a mercury manometer, thus permitting continuous visualization of the mean blood pressure and arterial pulsation. Each dog was dialyzed for 3 hours at an average flow rate of 150 ml per minute. The volume of the bath fluid was arbitrarily fixed at 50 L in the ethanol experiments and at 100 L in the cases of methanol-poisoned animals; its composition was as usually made for clinical dialysis and the temperature was kept constant at 39° C.

Chemical determinations of ethyl alcohol were done by reduction of acid potassium dichromate according to the method described by Kingsley and Current (2). This technique in our hands yielded recoveries ranging from 95.6 to 101 per cent. The same reaction applied to aqueous solutions of methanol showed a strict accordance to the Lambert-Beer's law and a comparable degree of accuracy in recovery experiments. Thus, our dealing with one alcohol at a time in the *in vitro* experiments prevented any cumulative reducing effect and permitted the use of the same method for the quantification of methanol. However, because of the possible occurrence of interfering substances in biological material, analyses of plasma methanol were done by a specific microdiffusion technique using potassium carbonate as a liberating agent and potassium permanganate and chromotropic acid as reagents (3). Prior to analysis, all the plasma samples were found negative on testing for acetone and formaldehyde.

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[1] $D = a\frac{A - V}{A - B}$ in which D = dialysance (milliliters per minute), a = flow rate (milliliters per minute), A = arterial concentration (e.g., milligrams per 100 ml), V = venous concentration (e.g., milligrams per 100 ml), B = bath concentration (e.g., milligrams per 100 ml). This relates "clearance" in the artificial kidney to the flow rate, the arteriovenous difference across the dialyzer, and the blood-bath concentration gradient.

[2] Supplied by Travenol Laboratories, Morton Grove, Ill.

TABLE I

In vitro dialysis of ethanol

Coil no.	Flow rate	Inflow conc.	Outflow conc.	Bath conc.	A-V*	Dialysance
	ml/min	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*ml/min*
1	306	682	368	145	314	178
	134	464	304	201	160	81
	66	360	252	219	108	51
2	483	712	450	42	262	188
	368	321	235	89	86	136
	251	229	166	86	63	110
3	342	561	362	20	199	125
	246	434	228	26	206	124
	152	590	180	45	410	114
	100	419	145	85	274	82
4	507	439	304	32	135	167
	300	394	211	35	183	152
	210	319	139	66	180	149
	120	302	96	84	205	95
	70	247	76	66	170	66
5	470	492	366	62	126	138
	294	421	248	81	173	149
	228	356	181	86	175	142
	111	356	131	103	225	99
	55	271	105	82	166	48
6	480	628	422	60	206	174
	438	508	384	79	124	127
	393	462	332	94	130	139

* Arteriovenous difference across the artificial kidney obtained by subtracting the outflow concentration from the inflow concentration.

The dialysance was calculated by the formula of Wolf, Remp, Kiley and Currie (4), as shown in Footnote 1.

RESULTS

The results of the *in vitro* dialyses are presented in Tables I and II.

The dialysance of ethanol has been determined over a selected range of flow rates from 55 to 507 ml per minute. The experimental values obtained ranged between 48 and 188 ml per minute. Their relationship is shown graphically on Figure 1. A steep elevation of the dialysance is apparent with flow rates increasing to a value in the vicinity

TABLE II

In vitro dialysis of methanol

Coil no.	Flow rate	Inflow conc.	Outflow conc.	Bath conc.	A-V*	Dialysance
	ml/min	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*ml/min*
1	528	324	229	14	95	162
	418	256	181	20	75	133
	329	237	134	23	103	158
	182	216	93	33	123	122
	98	192	66	35	126	79
2	384	278	176	22	102	153
	146	184	84	33	100	96
	84	150	38	39	112	84
3	439	394	235	19	159	186
	370	318	181	26	137	174
	310	262	148	40	114	159
	230	208	116	46	92	130
	64	183	58	53	125	62
4	475	350	220	27	130	191
	413	290	178	36	112	182
	360	232	154	46	78	151
	268	184	111	52	73	148
	79	158	67	60	91	73

* See footnote to Table I.

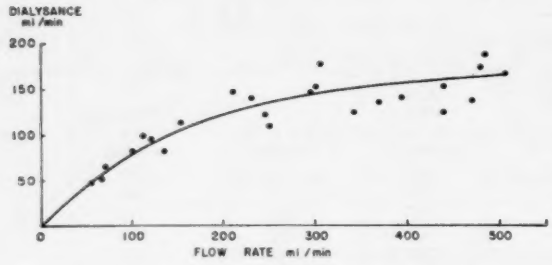

FIG. 1. DIALYSANCE OF ETHANOL *IN VITRO*.

of 225 ml per minute. Further increase of the flow, however, affected the removal of alcohol to a much lesser extent.

Similar results were obtained when dialyzing methanol at flow rates varying from 64 to 528 ml per minute. The dialysance of this compound ranged between 62 and 191 ml per minute and behaved in a comparable fashion with progressive elevation of the flow rate (Figure 2).

These data illustrate clearly the similarity of transfer of ethyl and methyl alcohol across the dialyzing membrane. The high dialysance of the two compounds compares favorably with that of urea as measured under similar conditions in the twin-coil kidney by Kolff and Watschinger (5) and Schreiner, Maher and Marc-Aurele (6) as well as that obtained from human dialyses by Meyer (7). Comparison with other non-ionized substances (6) revealed that at a flow rate of 200 ml per minute, the clearance of ethanol or methanol approximated 1.5 times that of uric acid, 1.2 times that of creatinine and 1.7 times that of phenobarbital.

Typical results obtained from *in vivo* experiments are illustrated in Figures 3 and 4. For simplicity of presentation, the plasma alcohol curve of only one control animal has been plotted in each graph. The data pertinent to each period of hemodialysis are summarized in Table III.

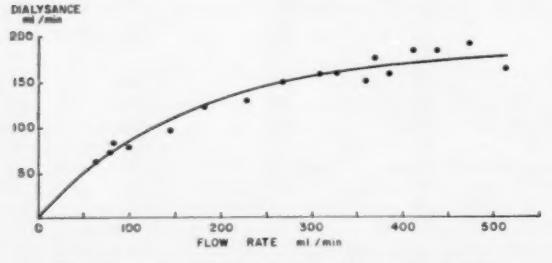

FIG. 2. DIALYSANCE OF METHANOL *IN VITRO*.

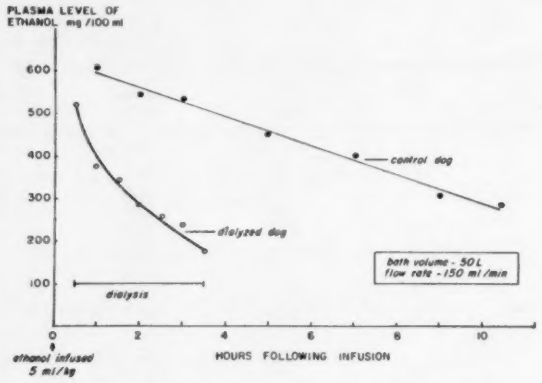

FIG. 3. EFFECT OF HEMODIALYSIS ON ACUTE ETHANOL INTOXICATION IN DOGS.

Figure 3 shows the plasma alcohol concentrations of two dogs infused with 5 ml per kg of absolute ethanol. The spontaneous decline of the plasma ethanol levels in the control animal was from 605 to 282 mg per 100 ml over a period of 10.5 hours. Three hours of hemodialysis lowered the plasma concentration of the other animal from 519 to 178 mg per 100 ml. Comparable results were obtained from another set of animals given 6 ml per kg of absolute ethanol (Table III).

Because of the slower metabolism of methyl alcohol, dogs poisoned with this compound were observed over a period of 48 hours. Figure 4 exemplifies the capability of the artificial kidney in removing methanol from an animal which had been given 5 ml per kg of the absolute compound. By the end of the third hour of the procedure, the dialyzed dog had its plasma concentration de-

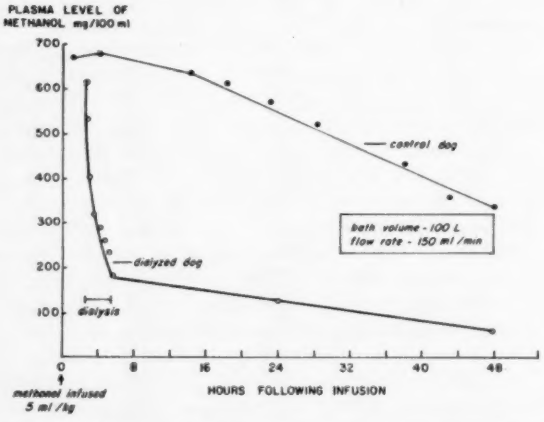

FIG. 4. EFFECT OF HEMODIALYSIS ON ACUTE METHANOL INTOXICATION IN DOGS.

creased from 615 to 180 mg per 100 ml. This contrasted with the control dog which still had a plasma level of 343 mg per 100 ml two days after the infusion. A similar contrast appears when comparing the data obtained from two other animals studied under identical conditions (Table III).

With two exceptions, each control was given the same amount of alcohol per unit of weight as its dialyzed partner (Table III). The efficiency of hemodialyses is thus reflected from a comparison of their respective hourly fall of plasma alcohol expressed in percentage of the initial level shown on the graphs. Such calculations disclosed that in the actual conditions, the artificial procedure was 4.2, 4.3 and 3.8 times faster than the physiological removal of ethanol by the control animals. In the cases of methanol poisoning the comparative efficiencies were 22 and 16 times that of the controls. Identical results from different sets of experiments can hardly be expected, considering the individual variations of the rate of metabolism of alcohol (8) as well as the individuality in coil performance (9). Further increase in the amount dialyzed would have been achieved with a higher flow rate or by changing the bath solution at the middle of each run (4).

The amount of ethanol recovered in the bath at the end of each *in vivo* dialysis was 29 and 33 per cent of the administered doses. Values of 25 and 27 per cent were found for methanol. This, however, represents strictly *minimum* recoveries, since no correction could be induced for the substantial loss of alcohol evaporated from the surface of the baths heated to 39° C.

Under clinical observations, all the ethanol control animals remained unconscious and unresponsive during the ten hours of study, in contrast to the dialyzed dogs which awoke by the second hour of the procedure.

Methanol-poisoned controls exhibited inability to stand on their feet for 24 hours and had a very ataxic gait for some 36 hours following the infusion. They all developed blindness with severe papillitis and retinal edema which subsided a few days later. Slight hyperemia of the optic disc was observed in one of the dialyzed animals, but blindness was never apparent in the dialyzed

TABLE III

Plasma alcohol concentrations in dogs submitted to three hours of hemodialysis as compared to nondialyzed control animals *

Dog	Wt.	Alcohol	Dose	Interval between end of infusion and dialysis	Plasma level (mg/100 ml)						Late blood samples	
					Minutes* 0	30	60	90	120	150	180	Time from infusion	Plasma alcohol concentration
	kg		*ml/kg*	*min*								*hrs*	*mg/100 ml*
B (Dialyzed)	22.7	Ethanol	5	30	519	377	343	283	258	234	178		
Control 1	13.1	Ethanol	5			605		549		535		10.5	282
Control 2	14.1	Ethanol	5			404		389		352		9	209
BWF (Dialyzed)	27.2	Ethanol	6	55	634	486	451	379	318	284	233		
Control 3	13.6	Ethanol	6			603		552			504	10	308
L (Dialyzed)	22.2	Methanol	5	190	535	445	343	298		245	238		
Control 4	17.7	Methanol	5		560						554	48	343
Control 5	13.6	Methanol	3.2		375						342	48	64
T (Dialyzed)	23.1	Methanol	5	145	615	405	320	290	260	233	180		
Control 6	13.1	Methanol	5		678						680	48	343
Control 7	14.0	Methanol	3.2		366						354	48	163

* Minutes after start of dialysis.

animals and both of them were alert at the end of the procedure.

DISCUSSION

More than 1,000 deaths are reported each year in the United States from acute alcohol poisoning (10). Massachusetts alone reported 4,505 deaths from ethyl alcohol and 118 from other alcohols in the period from 1928 through 1937. In a series reported from the Boston City Hospital (11), 59 per cent of all patients admitted in coma were suffering from severe alcoholic intoxication. The relationship of alcohol blood levels to clinical symptoms is succinctly outlined by Victor and Adams (12), who also give a good account of the history of alcohol poisoning. The metabolism and toxicity of methanol have been reviewed by Røe (13).

Methanol poisoning usually occurs in group outbreaks in young individuals (14–17), but methanol may even be poisonous by inhalation (18, 19). In addition to the danger of death, severe ocular damage represents a tragic form of late morbidity (20, 21).

Treatment of these poisonings has to date been symptomatic and supportive. Delineation of the criteria for dialyzability of circulating poisons (1) led us to postulate that ethanol and methanol would represent practical substances for dialytic removal. The present study represents experimental verification of these considerations. *In vitro* experiments show clearly the rapid removal rate of both alcohols. *In vivo* experiments confirm the high dialysance from biological solutions and demonstrate the ability to prevent or reverse coma.

Preliminary studies with C^{14}-labeled ethanol permitted simultaneous measurement of both alcohols and do not suggest any depressing effect of one upon the dialysance of the other. It would thus be rational and safe to use ethanol to block the metabolism of methanol in a poisoned patient. Since the metabolites of methanol are said to be 6 and 30 times as toxic as the parent compound (17), early dialysis provides a unique opportunity for preventive medicine. No direct measurements of formic acid or formaldehyde were made, but they too should be theoretically dialyzable.

SUMMARY

The dialysances of ethyl and methyl alcohol have been measured *in vitro*.

Data from experiments on dogs poisoned with ethanol or methanol have been presented. These provide a rational basis for the use of this technique in massive ethanol or methanol intoxications which are major clinical causes of personal and epidemic poisonings.

Emphasis is placed on the importance of early action with the artificial kidney in the treatment of acute poisoning.

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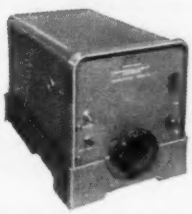

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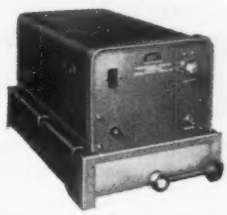

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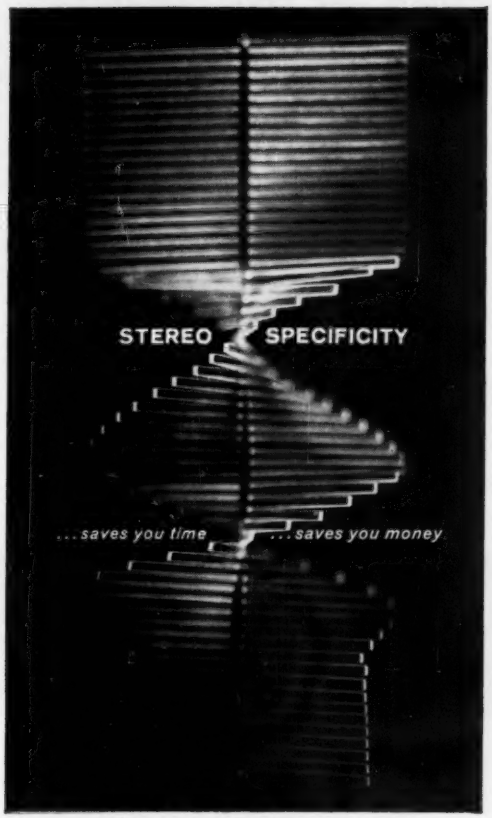

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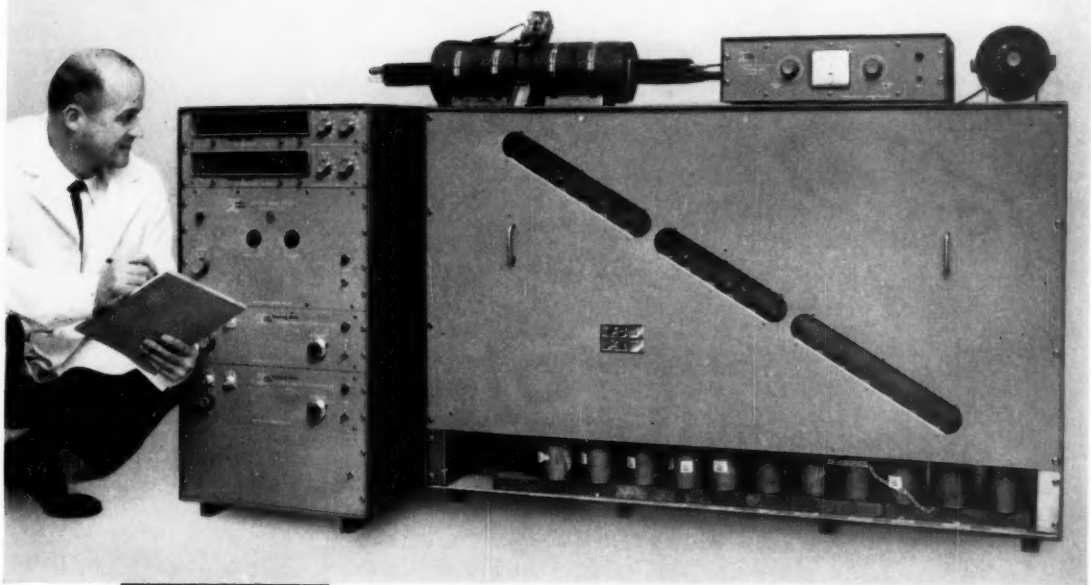

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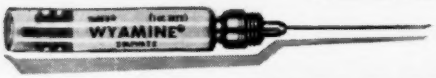

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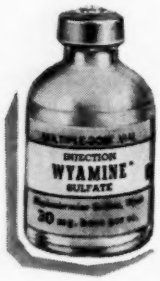

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